# *Actinobacillus lignieresi*; a study of the organism and its association with its hosts.

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Summary of a thesis presented for the degree of Doctor of Veterinary Medicine and Surgery of the University of Edinburgh

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SUMMARY

A survey of the literature has shown that Actinobacillus lignieresi has not been well characterised, its identification being dependent, in part, upon its association with typical actinomycotic lesions. Because of this, the existence of the commensal form of the organism has been suspected but never proven.

The morphological, cultural and biochemical characters of 220 strains of A. lignieresi isolated from pathological material have been investigated. A distinctive feature was the production of granules which, in conjunction with the bacilli themselves, gave a characteristic arrangement which has been named the "Morse code" form. A number of fermentable substrates were constantly fermented and others were consistently not attacked, whilst several substrates gave varying results with different strains. Several biochemical tests which have been shown to give constantly negative or positive results, can also be used for identification. An hitherto unrecorded character of A. lignieresi that has been demonstrated is the ability of many strains to synthesise starch from dextrose and maltose.

The antigenic structure of 218 strains of A. lignieresi was investigated by slide and tube agglutination tests and absorption tests. Six antigenic types (no. 1-6) and two subtypes (1a, 4a) of organisms were distinguished by differences in their heat-stable antigens; 203 of the strains were classified in these types and 15 were untyped. The majority of strains isolated from cattle belonged to type 1 and most of those from sheep to types 2, 3 and 4.

Heat labile antigens common to different antigenic types were found in living and formaldehyde-killed organisms. These antigens may be responsible for inagglutinability of living organisms tested with antisera to the heat-stable antigens. The heat-labile antigenic material appeared to be associated with extracellular slime produced in small amounts by the organism.

A medium containing oleandomycin and nystatin has been developed for the isolation of actinobacilli from mixed bacterial populations. Its use resulted in the isolation of organisms resembling A. lignieresi in morphological and biochemical characters from the ruminal contents of normal cattle and sheep and from

the tongues of normal cattle. The organisms from normal animals showed an antigenic relationship with the pathogenic strains.

Antibodies to A. lignieresi have been demonstrated in sera from normal adult cattle in titres up to 1 in 160. Very young calves, however, were shown not to possess such antibodies but to acquire them gradually during their first year of life. Antibodies to A. lignieresi in the sera from normal sheep were found at slightly higher levels than in cattle.

The value of the agglutination test as a diagnostic procedure for clinical actinobacillosis has been investigated. Most sera from clinically affected cattle and from slaughterhouse cases of the disease showed higher levels of antibody than normal animals and the occurrence of a prozone in tests with such samples was a notable feature which was absent in tests with sera from normal animals.

The relationship of Bacterium equirulis (Actinobacillus equuli) to A. lignieresi has been investigated. Similarities were apparent in the morphological, cultural, biochemical and antigenic characters.

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FOREWORD

Actinobacillus lignieresi, recognised for more than half a century as a pathogen of cattle and latterly identified as a pyogenic organism of sheep, has been suspected of having a commensal role in these animals, but its presence in normal animals has not been detected. This is due partly to the fact that the identity of the organism has been established in some degree by its association with the characteristic lesion, and partly that the intrinsic characters (biochemical, fermentative and antigenic properties), which might be used more satisfactorily for identification, have been examined in only small numbers of strains of the organisms, so that variations in these characters have often led to divergent and confusing results.

The diagnosis of typical actinobacillosis of the tongue in cattle is usually made on clinical grounds and presents no great problems, but actinobacillosis of other parts of the bovine alimentary tract may lead to difficulties in differential diagnosis. No satisfactory bacteriological or serological test is available for this purpose.

It has been the intention in this work firstly to

examine a large number of strains of A. lignieresi isolated from known pathological material to determine the morphological, cultural, biochemical and antigenic characters of the pathogenic organism, and thus establish it as a recognisable entity in the absence of the lesion; secondly, to investigate the possible existence of the organism in a commensal form in cattle and sheep; thirdly, to assess the feasibility of a diagnostic serological test for the disease in cattle; and lastly to compare A. lignieresi and Bacterium equirulis, organisms considered by some workers to be taxonomically related.

GENERAL INTRODUCTION

Actinobacillus lignieresi was first described by Lignieres and Spitz (1902) who isolated a small Gram-negative bacillus from actinomycotic lesions in cattle in Argentina. They referred to this organism as "the actinobacillus" but it was later named A. lignieresi in honour of Lignieres (Brumpt, 1910).

Actinobacillosis in cattle most often takes the form known as "wooden tongue", a chronic granulomatous lesion occurring in the tongue (Bosworth, 1923; Davies and Torrance, 1930; Vawter, 1933; Cavandoli, 1945; Collak and Orsag, 1957-8; Till and Palmer, 1960) as a result of which the tongue becomes hardened and fibrosed, causing interference with prehension and mastication. Lesions are also seen frequently in the lymph glands associated with the tongue (Thompson, 1933a), often being found here in the absence of any detectable lesions in the tongue itself. Another form of the disease in cattle may be found affecting the superficially situated lymph glands of the head resulting in a suppurative swelling which may burst through the skin (Angelucci, 1933; Lucido, 1936; Robinson, 1951; Sanders and Ristic, 1956). Lesions are frequently found in the cheek, gums and palate

(Griffith, 1916; Davies and Torrance, 1930; Thornton, 1943; Till and Palmer, 1960). The bones of the head, particularly the mandible, may become involved in the pathological process by direct extension from lesions involving the soft tissues, producing changes which are very similar, at least macroscopically, to those seen in actinomycosis of the jaw caused by Actinomyces bovis. Such bony changes due to Actinobacillus lignieresi have been recorded by Bosworth (1923), Damman (1927), Bruere (1955) and Whittem and Pearson (1963). That the most frequent lesion in actinobacillosis of the bovine animal involves the structures of the head is borne out by the incidence of such lesions in slaughterhouse material, between 47.6 and 76.8 per cent. of those animals slaughtered at the Edinburgh abattoir and showing lesions of actinobacillosis having had lesions confined to the head (Edinburgh Medical Officer of Health, 1955, 1956, 1957, 1958, 1959, 1960, 1961 and 1962).

Other parts of the alimentary tract may be involved in the infection, particularly the oesophagus, rumen and reticulum (Davies and Torrance, 1923; Bruere, 1955). Actinobacillosis of the pharynx has been recorded by Bosworth (1923) and Cordy (1949).

The respiratory tract may be involved in the disease, lesions having been described in the nasal cavity (Barboni, 1938; Cordy, 1949), the trachea (Bertellotti, 1942), the lungs (Davies and Torrance, 1930; Sforza, 1941; Cavandoli, 1945; Cordy, 1949) and the pleura (Misdorp, 1963). Lesions of the liver have been reported (Davies and Torrance, 1930).

Subcutaneous lesions in parts of the body other than the head have been described in some cases involving the skin and/or underlying subcutis (Palotay, 1951; Hebeler, Linton and Osborne, 1961; Mawditt and Greenham, 1962), whilst in others the associated lymph glands have also been affected (Damman, 1927; Gerring, 1947). Skeletal muscles may be involved in the pathological process and Mawditt and Greenham (1962) reported such a lesion in their case of subcutaneous actinobacillosis.

Actinobacillosis of other organs and tissues have been encountered from time to time, but their frequency is low. Menzani (1933) described changes of the vulva, vagina and cervix which he ascribed to A. lignieresi, whilst lesions of the penis (Pou, 1937) and testicle and ovary (Palotay, 1951) have been reported. Mammary actinobacillosis has been observed

and diagnosed clinically, but was not confirmed (Potter, 1942; Dawson, 1945).

Bovine actinobacillosis has a world-wide distribution, reports having been made of its occurrence in Argentina (Lignières and Spitz, 1902; Cavandoli, 1945), Australia (Pullar, 1939; Ropert, 1942; Gerring, 1947; Whittem and Pearson, 1963), Canada (Damman, 1927), Czechoslovakia (Čollák and Orsàg, 1957), Eritrea (Sforza, 1941), Great Britain (Griffith, 1916; Bosworth, 1923; Till and Palmer, 1960), India (Mangrulkar, 1939), Italy (Angelucci, 1936; Barboni, 1938; Bertellotti, 1942), Kenya (Daubney, 1941), New Zealand (Linton, 1946; Bruere, 1955), South Africa (Robinson, 1951), Uruguay (Pou, 1937), U.S.A. (Thompson, 1933a; Vawter, 1933) and Yugoslavia (Smrěck, 1952).

It was not until nearly 30 years after the original description of A. lignieresi by Lignières and Spitz that 2 cases of actinobacillosis in sheep were first recorded (Bosworth, 1927). Thomas (1931) described a more extensive outbreak of actinobacillosis in sheep and in the same year Bosworth and Glover (1931) pointed out the close similarities in the clinical conditions in sheep brought about by the

actinobacillus and by an organism which had been named Bacterium purifaciens by Christiansen (1917). Marsh and Wilkins (1939), describing an outbreak of actinobacillosis in a flock of sheep, stated that they were of the opinion that this was the same disease as that caused by Bact. purifaciens, and a similar opinion was expressed by Wramby (1940). Tunnicliff (1941) and Taylor (1944) made comparisons of strains of A. lignieresi from cattle and Bact. purifaciens from sheep and both workers came to the conclusion that the two organisms are identical.

The disease in sheep differs from that in cattle in that the most frequent site of the lesion is not the tongue but the skin of the head. The fibrosis which is a feature of the lesions in cattle is not so apparent in sheep, the infection giving rise to markedly suppurative changes, with so-called "plastic pus" to which the name pyobacillosis has been applied. The most frequently described sites of lesions are the face (Ravaglia, 1934; Marsh and Wilkins, 1939; Taylor, 1944) and the lips (Davis and Stiles, 1939; Wramby, 1940). The lymph glands of the head may be extensively involved (Thomas, 1931; Davis and Stiles, 1939; Wramby, 1940; Hayston, 1948) and Taylor (1944)

noted that "the demonstration of normal lymphatic tissue in the head may prove difficult, the lymph nodes being almost totally replaced by abscesses of varying size . . . ". Lesions have been described in sites other than the head. Jowett (1931) considered the commonest site to be the lung in slaughterhouse material, and such lesions were also recorded by Magnusson (1929). The mediastinal and bronchial lymph glands were involved in the cases described by Thomas (1931) who also observed lesions in the abomasum. The liver and kidneys have also been observed as the seats of lesions (Magnusson, 1929; Jowett, 1931) and Magnusson also described a case in which the udder was affected. Johnston (1954) recorded a case of nasal actinobacillosis involving the mucosa overlying the nasal septum. Taylor (1944) noted that advanced cases of the disease involving the tissues of the head may show changes of the bones with marked rarefaction and exostoses similar to those seen in cattle.

The geographical distribution of ovine actinobacillosis is extensive with workers having recorded its existence in Australia (Hayston, 1948), Denmark (Christiansen, 1917), Great Britain (Jowett, 1931; Taylor, 1944), Holland (Dekker, 1957), Italy

(Ravaglia, 1934), Norway (Thorshaug, 1934), South Africa (Thomas, 1931), Sweden (Magnusson, 1929) and U.S.A. (Davis and Stiles, 1939).

The differentiation between actinomycosis (Actinomyces bovis infection) and actinobacillosis (Actinobacillus lignieresi infection) may not be readily made on macroscopic or even general histological grounds and this has led to some confusion of terms in the earlier literature, the term actinomycosis being used generally to include infections with the actinobacillus. M'Fadyean (1932) pointed out that the differentiation could be made quite easily by microscopical examination of the lesion to demonstrate the morphology and staining reactions of the causal organism. It is worthy of note that cases have been described in which organisms other than A. lignieresi have been isolated from lesions resembling actinobacillosis in cattle. Hugo (1962) described an outbreak of abscesses associated with A. lignieresi and Corynebacterium pyogenes infection. Slavin (1944) examined subcutaneous lesions, surgically removed from the necks of cattle, which histologically appeared as typical of actinobacillosis. The organism recovered from these lesions, however,

differed from A. lignieresi in a number of its characters. Actinobacillosis-like lesions in cattle and sheep, from which Bacillus subtilis was isolated, were described by Schweiger, Trainer and Eveleth (1943). These lesions were present in the liver, mandibular region or lung, and same cases were seen in which there was involvement of the bony structures of the head. It is also of interest that proven cases of actinobacillosis may not always present the typical features of the disease. Misdorp (1963) drew attention to lesions in the lungs and pleura of cattle observed at meat inspection, which had similarities to tuberculosis.

The occurrence of actinobacillosis in animals other than cattle and sheep has been reported from time to time, but the organisms recovered have not always been identified as A. lignieresi. Bucy (1948) described the clinical signs and course of a disease in a mare, which he had diagnosed as actinobacillosis. No bacteriological examination of material taken from the case was made and whether the diagnosis was correct or not remains in doubt.

In the dog actinobacillosis of the tongue has been recorded by Fletcher, Linton and Osborne (1956)

and an organism of the Actinobacillus group isolated. These authors noted slight differences between their organism and A. lignieresi, but Kemenes and Markoi (1959) isolated a coccobacillus from a sublingual swelling in a dog and identified this as A. lignieresi. Savage and Isa (1956) reported a granulomatous lesion from the trachea of a dog which they diagnosed as "actinomycosis" but in which the organisms were Gram-negative. No cultural examination was carried out.

Infection of human beings with A. lignieresi is of infrequent occurrence but has been reported. Ravaut and Pinoy (1911) isolated an organism from the cerebro-spinal fluid of a 16-year-old boy with meningitis and considered this to be identical with the organism described by Lignières and Spitz. Their diagnosis was confirmed by Lignières himself. A fatal case of meningitis in an 11-month-old boy was attributed to an organism of the Actinobacillus group (Gerdine and Pease, 1926). Thompson and Willius (1932) believed that a febrile disease in a butcher was actinobacillosis which they considered to be associated with the patient's occupation and the fact that he had cut himself while at work. Thompson and

Willius isolated a Gram-negative bacillus which was identified as A. lignieresi from blood cultures, and repeated isolations were made over a period of 3 months (Lawrence, Neuhauser and Howell, 1932). Beaver and Thompson (1933), investigating a fatal disease in a man, observed granulomatous abscesses in the lungs, liver and spleen, and commented that the lesions were essentially similar to those of actinobacillosis in cattle. They isolated an organism which revealed a close cultural and antigenic relationship with A. lignieresi. More recently actinobacilli have been isolated from the sputum of patients with infected cervical lymph glands (Pathak and Ristic, 1962).

Several authors have reported the isolation of organisms which appear to belong to the Actinobacillus group. Arseculeratne (1961) described a naturally occurring infection in 3 laboratory rabbits, in which induration of the skin in the region of the tarsal joints occurred. Multilocular abscesses developed in the affected area and a Gram-negative bacillus, which was regarded as distinct from A. lignieresi, was isolated. Later, the name of A. capsulatus was proposed for this organism (Arseculeratne, 1962).

In rams, an organism isolated from the semen in

cases of epididymitis in Australia was considered to represent a new species of the Actinobacillus group, and it was named A. seminis (Baynes and Simmons, 1960). Livingston and Hardy (1964) in America isolated an organism which they believed to be identical with the Australian isolate.

Bacterium equirulis, an organism associated with pyaemic infections of horses, is considered to be a member of the genus Actinobacillus and has been named A. equuli (Breed, Murray and Smith, 1957). This organism was isolated from verrucose endocarditis in a pig by Ashford and Shirlaw (1962), and an account of an acute fatal infection in piglets was given by Zimmermann (1964) who also described other organisms, isolated from piglets, with the characters of the genus Actinobacillus but differing from A. equuli. The pathogenic significance of these strains was not assessed, but the name of A. suis was proposed for them. This name had been used previously by van Dorssen and Jaartsveld (1962) for an organism isolated from the tissues of swine affected with an acute disease. The Dutch and German organisms differed from each other in their characters so that some taxonomic clarification is needed.

## Part I: The characters of *Actinobacillus lignieresi* recovered from pathological material

## 1. INTRODUCTION

In their description of actinobacillosis, Lignières and Spitz (1902) emphasised the similarities between this disease and actinomycosis, and described the characters of the organisms which they isolated from the pus obtained from lesions. The identity of the organism, based as it was on its morphological and cultural characters, was closely linked with its recovery from lesions, thus making the disease, and therefore the reaction of the host, one of the classifying features of the organism. Lignières and Spitz did not investigate the biochemical and fermentative activities of their organism except for the test for indole, its action upon glucose and lactose, and its mode of growth in urine. It remained for subsequent workers to examine these characters in detail (Bosworth, 1923; Magnusson, 1929; Davies and Torrance, 1930; Jowett, 1931; Taylor, 1944; Ristic, Herzberg, Sanders and Williams, 1956). As more investigators worked with A. lignieresi it became apparent that considerable variation existed in the properties of the organism, but satisfactory comparisons between the results of different workers were not always possible since the range of sugars

used in fermentation tests and the number of biochemical activities tested often varied from one investigator to another. Thus, Bosworth (1923) used 6 fermentable substrates and tested for indole production, whilst Taylor (1944) used 19 fermentable substrates although still only testing for indole production. Ristic et al. (1956), however, used only 9 fermentable substrates but carried out tests for the production of indole, nitrite from nitrate, urease, catalase and hydrogen sulphide. Another variable factor present in these investigations was the number of strains of the organism used by various workers, most of whom used only small numbers (1-6) (Ravaglia, 1934; Davis and Stiles, 1939; Johnston, 1954). Even when larger numbers were used they were limited to less than 30 strains, e.g. Bosworth (1923) 17, Davies and Torrance (1930) 16, Griffith (1916) 23, Ristic et al. (1956) 14, Taylor (1944) 10. These variable results have led Wilson and Miles (1946, p. 390 and 1955, p. 472) to the view that "The fermentative ability of this organism is a little doubtful.".

The identity of A. lignieresi isolated from a site other than a lesion (e.g. a commensal form of the organism) would be difficult to establish unless the

exact intrinsic characters of the pathogenic organism were known for comparison. It is the purpose in this section of the work to examine the morphological, cultural and biochemical characters of 220 strains of A. lignieresi isolated from lesions of actinobacillosis in cattle and sheep, and of 5 strains of A. lignieresi obtained from the National Collection of Type Cultures.

## 2. MATERIALS AND METHODS

### (a) Media

Except where otherwise stated the media used in this section of the work were prepared as described by Mackie and McCartney (1948).

### (b) Isolation of organisms from lesions

After searing with a heated spatula the surface of tissues showing macroscopic lesions, small portions of the infected tissue were withdrawn into sterile Pasteur pipettes for inoculation on horse blood agar plates and in glucose broth. The ease with which material could be obtained in this way varied. With material of bovine origin there were difficulties because of the small amount of pus and the fibrous nature of the lesions, whereas with material derived from sheep the quantity of pus was usually greater.

After incubation at 37°C for 18-24 hours, colonies having the characters for *Actinobacillus lignieresi* described by Wilson and Miles (1955) were picked into nutrient broth for further examination. In most cases *A. lignieresi* was obtained in pure cultures on the primary plates.

Whenever possible the nature of the lesion was confirmed by histological examination of sections stained by Gram's method and with haematoxylin and eosin.

(c) Preservation of stock cultures

Previous experience with a small number of strains of A. lignieresi maintained in the Department for class purposes had shown that this organism did not remain viable for more than a few days when grown on nutrient agar or blood agar. Each strain, as soon as possible after isolation, was dried in vacuo suspended in normal rabbit or normal horse serum (bovine and ovine sera were not used because of the possibility of antibodies being present in these susceptible animals). This method of preservation was found to be very effective and strains have remained viable for periods up to 10 years.

For day-to-day working, strains of A. lignieresi were maintained in cooked-meat medium in which they remained viable for up to 4 weeks.

(d) Examination for capsulation

Cultures grown on 0.1 per cent. and on 1.0 per cent. dextrose agar were examined for the presence of

capsules and extracellular slime by the wet-film India-ink method of Duguid (1951).

(e) Biochemical reactions

Each strain of A. lignieresi was subjected to the following tests:-

(i) fermentative activity
(ii) production of ammonia
(iii) production of indole
(iv) production of catalase
(v) production of hydrogen sulphide
(vi) reduction of nitrates
(vii) hydrolysis of urea
(viii) methyl red test
(ix) Vöges-Proskauer test
(x) methylene blue reduction test
(xi) ability to grow on MacConkey's medium.

(i) Fermentative activity. Organisms were grown in peptone water containing Andrade's indicator and 1 per cent. of the following substrates: dextrose, laevulose, mannose, galactose, arabinose, xylose, rhamnose, sucrose, maltose, lactose, trehalose, raffinose, inulin, dextrin, glycerol, mannitol, dulcitol, sorbitol, salicin and inositol. Incubation

at 37°C was continued for 14 days and readings made every day. The results with this medium were sometimes difficult to interpret since slight acid production did not give a very marked pink colour with the Andrade's indicator. Lovell and Hughes (1935), working with haemolytic coccobacilli recovered from the respiratory tract of calves, experienced the same difficulty which they overcame by using brom-thymol-blue indicator. Bosworth and Lovell (1944), investigating similar organisms isolated from sheep, employed a medium consisting of peptone water containing 10 per cent. broth with 7.5 per cent. brom-thymol-blue (B.D.H. indicator solution) with 1 per cent. of the fermentable substrate added. This medium was tried in the present work and, although the results obtained did not differ from those with Andrade's-indicator-peptone water, the interpretation was very much easier. Accordingly all strains were retested in the brom-thymol-blue sugar medium which was then used in subsequent sections of the work.

(ii) Ammonia production. Five-day-old peptone water cultures were tested for the presence of ammonia by the addition of Nessler's reagent. A positive reaction was indicated by the development of a brown colour and

a negative reaction by a faint yellow colour.

(iii) Indole production. The test for indole was carried out on 5-day-old peptone water cultures to 2-3 ml. of which 1 ml. of ether was added and shaken. After allowing the ether layer to separate, 0.5 ml. of Ehrlich's rosindol reagent (para-dimethyl-amido-benzaldehyde in acid alcohol) was added. The presence of indole was shown by a rose-pink coloration of the ether layer, but no colour change occurred with a negative test.

(iv) Catalase production. The organism was grown on a nutrient agar slope for 24 hours. One ml. of hydrogen peroxide solution (10 vol.) was run over the growth, a positive reaction being shown by the development of gas bubbles.

(v) Hydrogen sulphide production. The evolution of hydrogen sulphide was tested for with lead acetate paper inserted between the cotton wool plug and the tube containing the lactose peptone water in the fermentation tests. Hydrogen sulphide caused blackening of this paper. With negative results readings were made over the 14-day incubation period of the fermentation tests.

(vi) Reduction of nitrates. The medium used for this test was that described by Kauffmann (1954, p. 357), the organism being grown for 4 days at 37°C in the nitrate broth. The presence of nitrites in the medium was then shown by the addition of acid solutions of sulphanilic acid and ∝-naphthylamine, a red colour developing with a positive reaction.

(vii) Hydrolysis of urea. The urea medium of Christensen (1946) was used to detect the presence of urease activity. Cultures were incubated at 37°C for 14 days.

(viii) Methyl red (M.R.) test. The organism was grown for 4 days in glucose-phosphate-peptone water (Kauffmann, 1954, p. 358) at 37°C. The M.R. test was performed by adding 2-3 drops of 0.25 per cent. alcoholic solution of methyl red. A red colour developed with a positive reaction and a yellow colour with a negative result.

(ix) Vöges-Proskauer (V.P.) test. Cultures in the medium used for the M.R. test were incubated at 37°C for 4 days. The Barritt (1936) modification of the V.P. test was used in which 1 ml. of 6 per cent. alcoholic ∝-naphthol and 0.4 ml. of 40 per cent. caustic potash was added to 2 ml. of the culture and

shaken. A positive V.P. test was indicated by the development of a pink colour within 5 minutes.

(x) Methylene blue reduction test. This test was that described by Wilson and Miles (1955, p. 452). The organism was grown in nutrient broth at 37°C for 24 hours. One drop of 1 per cent. aqueous methylene blue solution was added to the culture which was reincubated at 37°C for 15 minutes. Complete decolorisation of the methylene blue was obtained with a positive test, a green colour indicated a weak positive reaction, whilst persistence of the original blue colour was seen with a negative result.

(f) Starch-forming properties

All strains of A. lignieresi were grown overnight on 1 per cent. dextrose and maltose agar slopes and the cultures then flooded with Gram's iodine diluted 1 in 10. Three reactions were obtained in this test:-

(a) bacterial growth coloured bluish purple (strong positive),

(b) bacterial growth coloured reddish brown (weak positive),

(c) bacterial growth not coloured or only slightly yellow (negative).

A small number of strains of A. lignieresi were tested for starch-production on nutrient-agar slopes containing 1 per cent. of the following "sugars": laevulose, galactose, xylose, sucrose, lactose, dextrin, mannitol, dulcitol, salicin and inositol.

## 3. RESULTS

### (a) Host and tissue sources

Two hundred and twenty-five strains of A. lignieresi were examined of which five were obtained from the National Collection of Type Cultures (no. 4189, 4191, 4975, 4976 and 4985); the remainder were isolated from bovine and ovine tissues and their anatomical and host sources are shown in Table 1 and individually in Appendix A. In 201 cases, where tissues were available for histological examination, the lesions presented the typical microscopical appearance of actinobacillosis, viz. a central bacterial colony staining Gram-negative, surrounded by a zone of "clubs" giving a daisy head appearance to the lesion; this in turn was surrounded by the granulomatous reaction characterising the tissue changes seen in actinomycosis and actinobacillosis.

In Table 1, strains isolated from the lymph glands draining the organ mentioned are included as though isolated from that organ. Of the strains isolated from "other tissues" in sheep, all except one were obtained from superficial abscesses either in the skin or lymph glands of the head and shoulder region. The

remaining ovine strain was isolated from a brain abscess. The strains recovered from bovine "other tissues" presented a more varied group of sources viz. skeletal muscle 1 strain, liver 3 strains, jaw 4 strains, lymph glands (site unknown) 4 strains and skin of throat (granulomatous lesion) 1 strain.

TABLE 1

Host-tissue distribution of A. lignieresi strains

| | Tongue | Lung | Rumen or Reticulum | Other Tissues | TOTAL |
|---|---|---|---|---|---|
| Sheep | 0 | 9 | 0 | 15 | 24 |
| Bovine | 174 | 3 | 6 | 13 | 196 |
| TOTAL | 174 | 12 | 6 | 28 | 220 |

(b) Morphological characters

All strains of A. lignieresi appeared as Gram-negative bacilli, but there was considerable variation in the length of the organisms from one strain to another. Some strains presented a distinct coccobacillary form (Fig. 1), whilst others showed longer rods (Fig. 2). The majority of strains showed a mixture of forms with the short rods predominating.

The morphology was found to vary with the medium

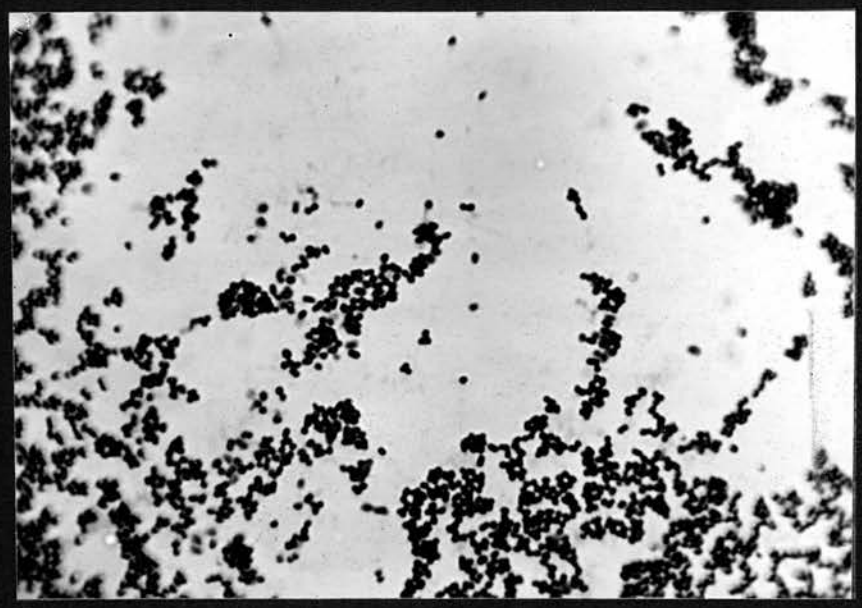

Fig. 1: 24-hour blood agar culture of A. lignieresi (strain A2) showing coccobacillary forms. Gram. X2000.


Fig. 2: 24-hour blood agar culture of A. lignieresi (strain A3) showing bacillary forms and many granules (a). Gram. X2000.

on which the organism was grown. On nutrient agar, blood agar and Loeffler's serum, the cultures showed a majority of short bacillary or coccobacillary forms (Fig. 3), but when the same strain was grown on dextrose or maltose agar much longer forms (Figs. 4 and 5) and even filaments (Fig. 6) were observed. The intensity of staining by Gram's stain showed variation between individual organisms within the same smear, some bacilli taking the stain well and others being only faintly coloured and having the appearance of "ghost" forms (Fig. 7). Bipolar staining was often apparent.

A constant feature of cultures of A. lignieresi on any medium was the occurrence of small granules scattered amongst the bacilli (Fig. 2). The granules varied in number, being more easily apparent in some strains than in others. They stained in the same way as the organisms and in most cases were not attached to the bacilli, although here and there an occasional granule could be seen attached or lying very close to a bacillus. This gave a picture which can be likened to the conventional representation of the letters of the Morse code (e.g. - · and - ··) (Fig. 8). Granules of this type were not seen in a large number of other

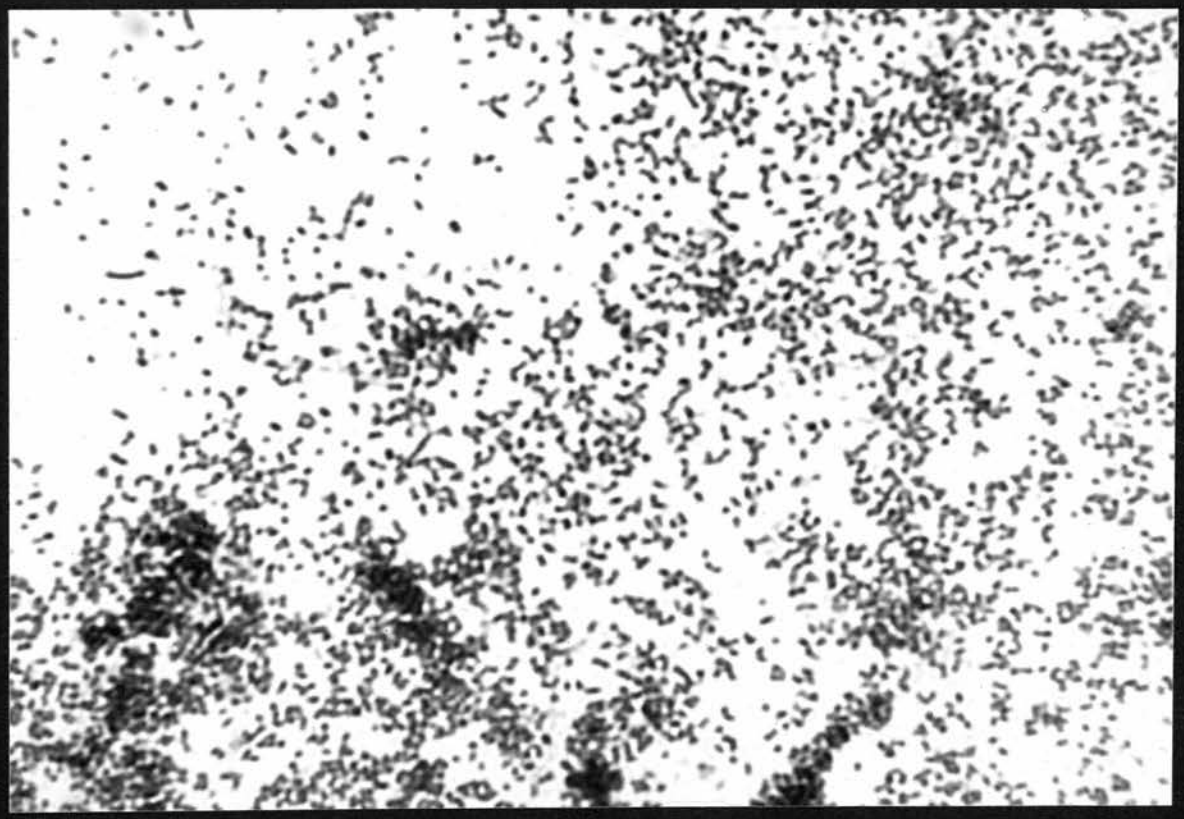

Fig. 3: 24-hour nutrient agar culture of A. lignieresi (strain A36) showing bacillary and coccobacillary forms. Gram. X2000.

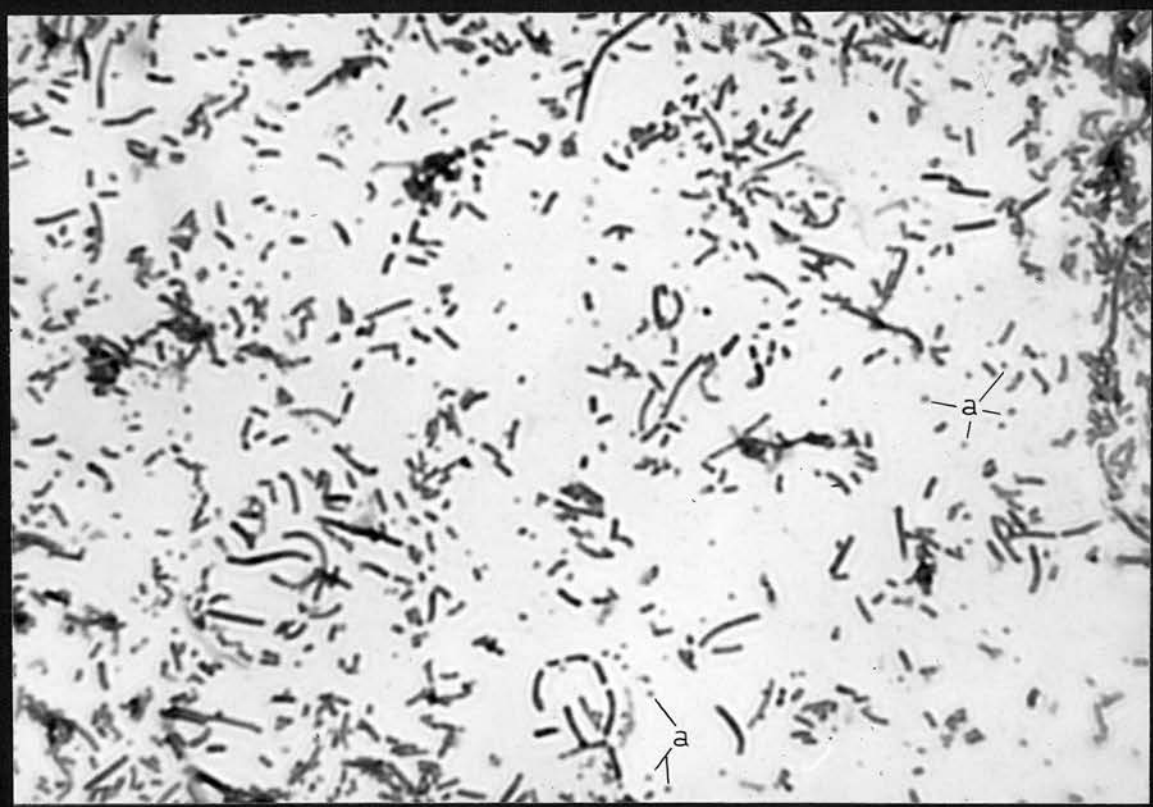


Fig. 4: 24-hour dextrose agar culture of A. lignieresi (strain A36) showing long bacillary forms and granules (a). Gram. X2000.

Fig. 5: 24-hour maltose agar culture of A. lignieresi (strain A36) showing long bacillary forms. Gram. X2000.

Fig. 6: 24-hour dextrose agar culture of A. lignieresi showing filamentous organism. Gram. X2700.

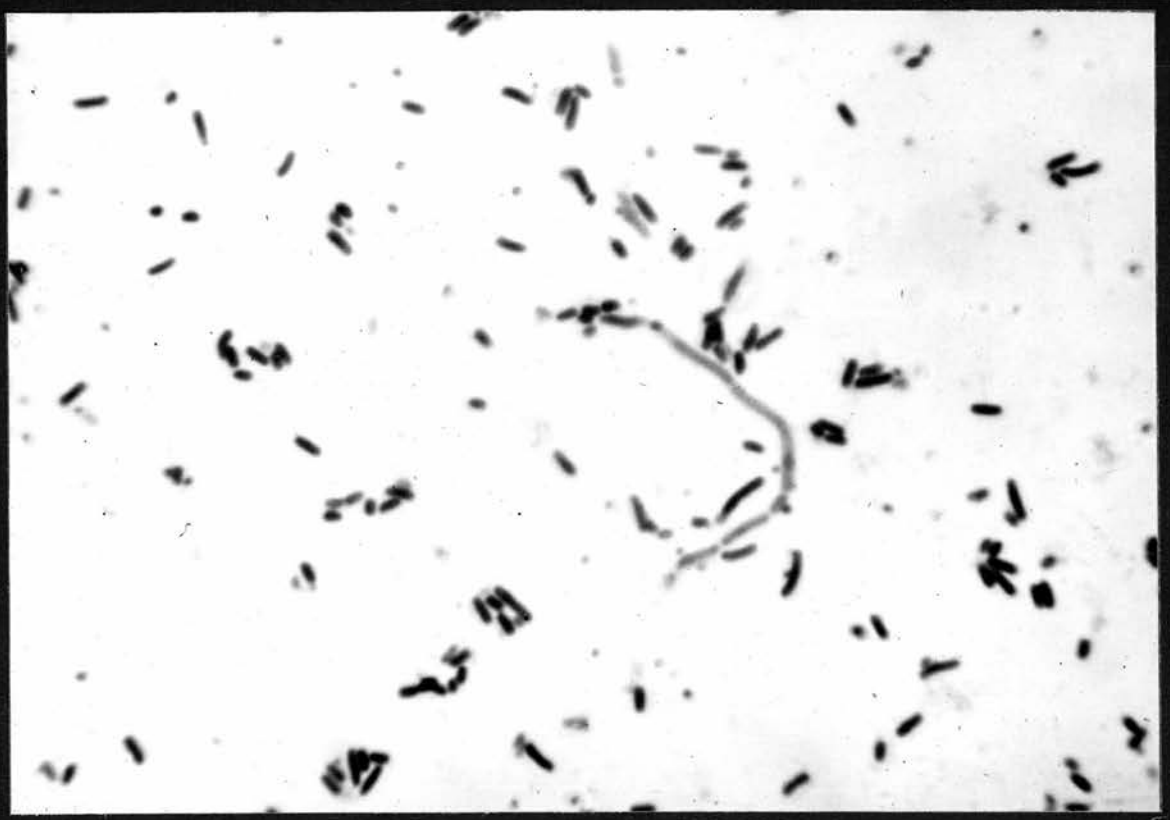

Fig. 7: 24-hour dextrose agar culture of A. lignieresi showing 2 "ghost" forms with bacillary forms stained more deeply. Gram. X2700.

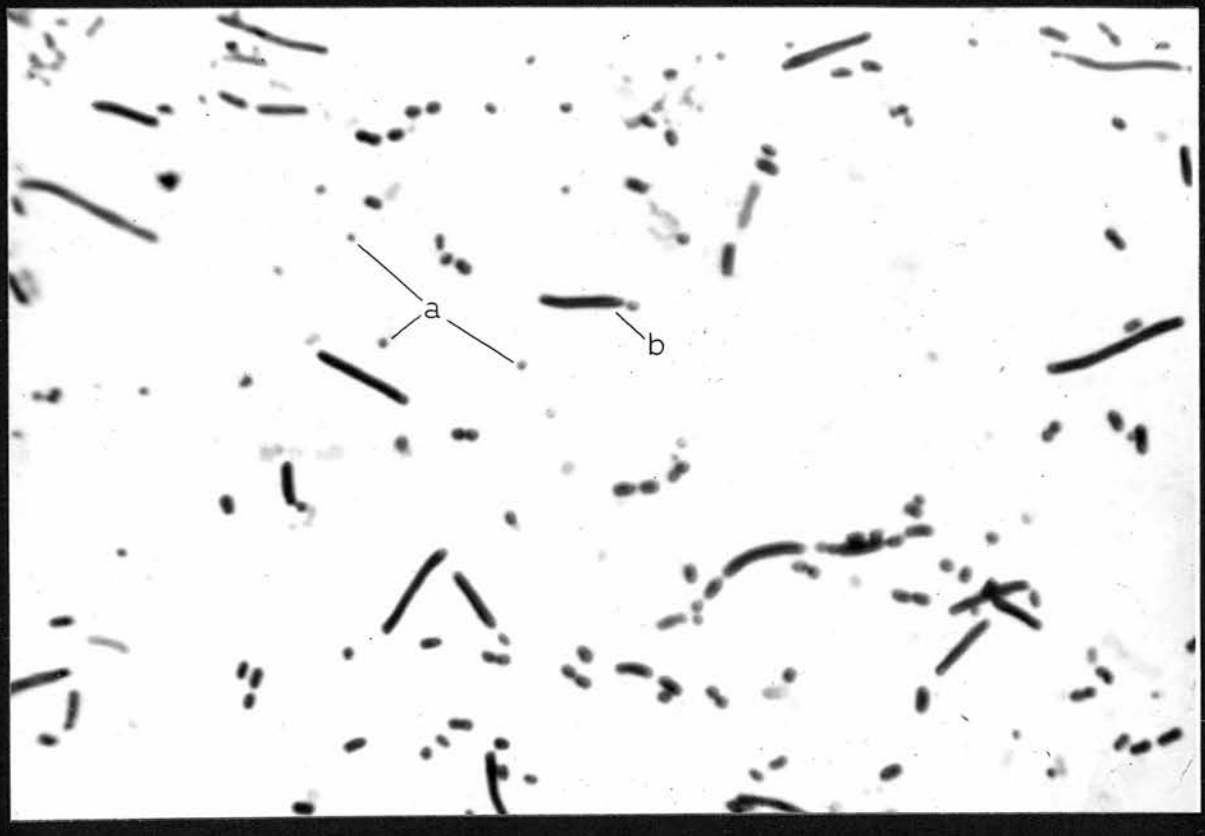


Fig. 8: 24-hour dextrose agar culture of A. lignieresi showing long bacilli, granules (a) and a "Morse code" form (b). Gram. X2700.

Gram-negative bacteria examined. The filamentous organisms were occasionally seen to break down into shorter bacillary forms interspersed with granules, giving an almost streptococcal appearance (Figs. 9 and 10).

A number of newly isolated strains of A. lignieresi were examined for the presence of capsules, but no evidence of these structures was found. There was evidence of the presence of extracellular slime, however, and it was shown that this substance was also produced by some strains which had not been freshly isolated from pathological material. Some evidence of such extracellular material was also seen in photographs of Gram-stained films of organisms under very high magnification (Fig. 11).

(c) Cultural characters

Colonies on blood agar were small (1-2 mm. diameter) after 24 hours and gradually increased in size with further incubation. No great variation in colony size was noted, although some strains were found which grew rather less profusely than most. This character was maintained even after repeated

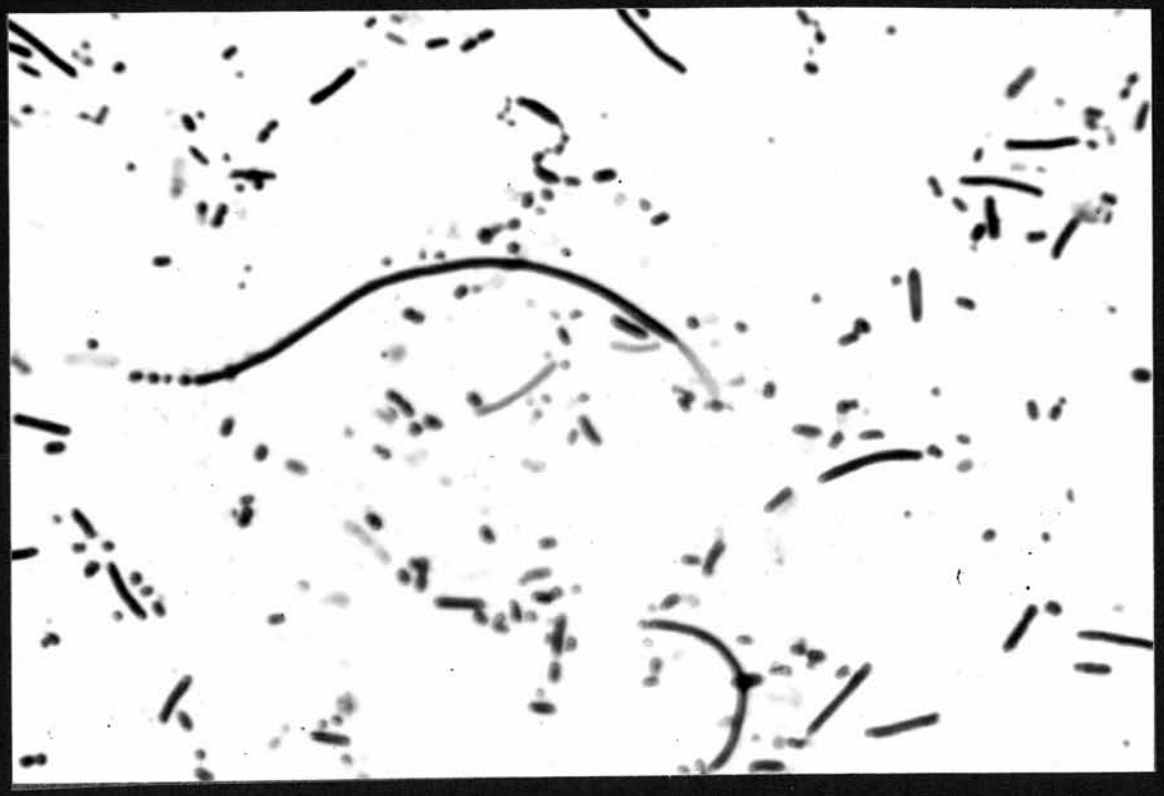

Fig. 9: 24-hour dextrose agar culture of A. lignieresi showing a filamentous organism broken down into granules at one end, with many scattered granules. Gram. X2700.

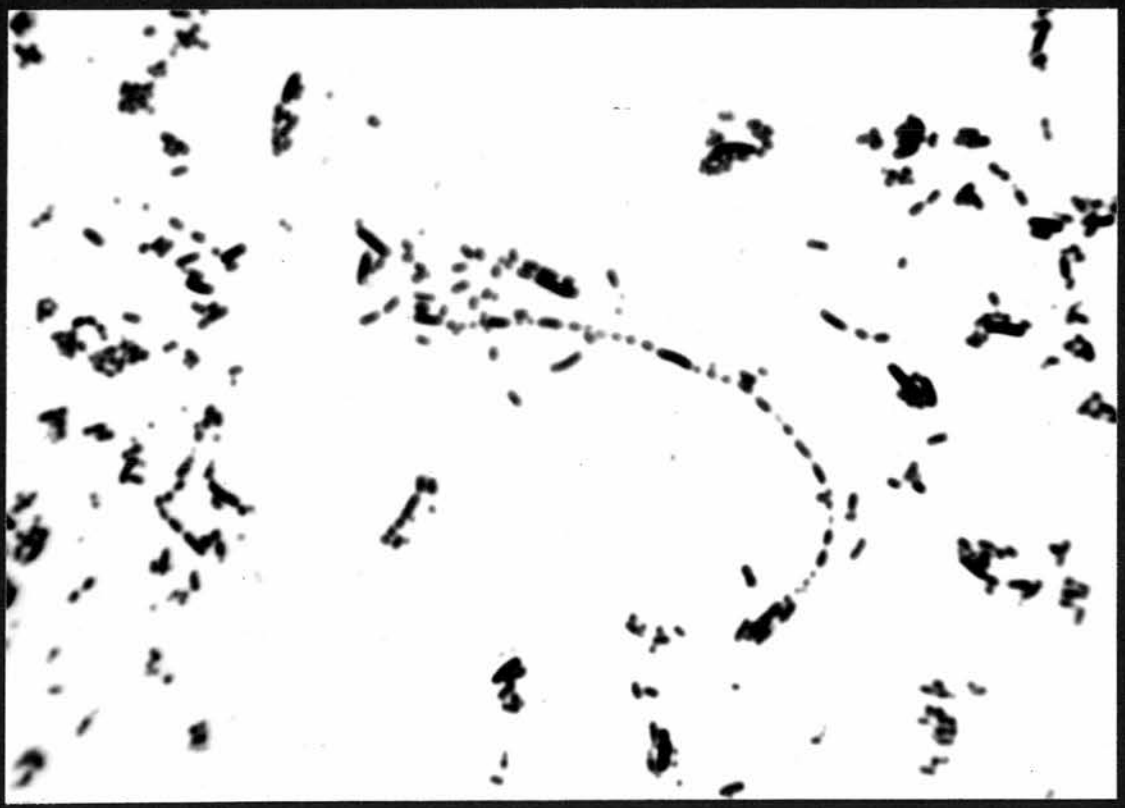

Fig. 10: 24-hour dextrose agar culture of A. lignieresi showing filamentous organism broken down into bacillary and granular forms. Gram. X2700.


Fig. 11: 24-hour blood agar culture of A. lignieresi (strain A2) showing evidence of extracellular slime (s) scattered between the bacterial cells. Gram. X4000.

subcultivation. Colonies on nutrient agar (infusion broth) were similar to those on blood agar, but on nutrient agar (Lab.-Lemco broth) growth was markedly poorer.

In most cases the colonies on blood and nutrient agar were viscous and difficult to remove completely from the surface of the medium. The adherence of the colony to the medium was most obvious in freshly isolated strains and usually disappeared after several subcultures, but the sticky nature of the colony often persisted in strains which had been repeatedly subcultured. The viscous nature of the bacterial growth was more marked in cultures grown on dextrose and maltose agar.

(d) Biochemical characters

The 225 strains fermented dextrose within 24 hours without the production of gas. There was also prompt (within 24 hours) fermentation of laevulose, mannose, xylose, maltose, dextrin and mannitol. No fermentation within 14 days occurred with trehalose, inulin, dulcitol, salicin and inositol. Differences between strains were noted in the reactions with the remaining substrates in the fermentation tests. The

differences for the 220 strains from pathological material and the 5 National Collection of Type Cultures strains are given individually in Appendix A and summarised in Table 2. More detailed figures of the number of strains isolated from pathological material giving various patterns of fermentation are given in Table 6 (p. 64).

TABLE 2

Fermentation reactions of A. lignieresi strains

| Substrate | Strains from pathological material | | N.C.T.C. Strains | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| Lactose | 185 | 35 | 2 | 3 |
| Sucrose | 217 | 3* | 5 | 0 |
| Sorbitol | 3 | 217 | 0 | 5 |
| Raffinose | 49 | 171 | 1 | 4 |
| Arabinose | 193 | 27 | 4 | 1 |
| Glycerol | 163 | 57 | 0 | 5 |
| Galactose | 123 | 97* | 4 | 1* |
| Rhamnose | 1 | 219 | 0 | 5 |

* Late fermentation

Lactose was fermented by 84.1 per cent. of the

strains derived from pathological material, but this occurred later than the first 24 hours of incubation, the majority of positive reactions appearing at 5 or 6 days with a range of 2-14 days. The proportion of N.C.T.C. strains fermenting lactose was less (40 per cent.), this discrepancy between N.C.T.C. strains and others being seen also in the fermentation of glycerol, where all N.C.T.C. strains failed to attack this substrate, whereas the majority (74.1 per cent.) of the strains from pathological material fermented it. Most strains were late in fermenting glycerol, the average time taken for this being 5 days.

Every strain, with the exception of 3 which did so between the 2nd and 5th days, fermented sucrose promptly. Similarly, all strains fermented galactose eventually, but 44.1 per cent. did so between the 2nd and 14th days. Of those strains which fermented raffinose and arabinose, most did so late, the average times for fermentation of these two substrates being 4 days and more than 7 days, respectively.

Although the results with sorbitol appeared to be quite distinct, 98.6 per cent. of strains from pathological material failing to ferment this sugar, it should be mentioned that, as this section of the work

was completed and the section related to commensal actinobacilli begun, discrepancies in the fermentation of sorbitol were noted. A small number of newly isolated strains of A. lignieresi (not included in these results) were found to ferment sorbitol as also were many of the actinobacilli isolated from bovine tongues and ruminal contents (see Part III, p. 103). Enquiries revealed that the batch of sorbitol which had been employed up to that time was almost used up and a new bottle had been opened for the preparation of the fermentation medium. Fortunately a small amount of the old sorbitol remained and it was possible to make comparative studies which led to the conclusion that strains of A. lignieresi which previously had failed to ferment sorbitol (old sample) now fermented this sugar (new sample). At the same time two other samples of sorbitol were obtained from other laboratories in the district and these gave the same results as the "new sample". All four samples were supplied by the same manufacturer, but the batch numbers on each container were different. The manufacturer was unable to give any explanation of the differences in microbiological activity exhibited by these samples of sorbitol but stated that all had been from the same source although

the "old sample" was about 12 years old. It was suggested that this sample "may have changed in some way with the passing of the years.".

Neither ammonia nor indole was produced by any of the strains of A. lignieresi examined and the M.R. test was negative in every case. All strains grew on MacConkey's medium and reduced nitrates to nitrites. With the remaining biochemical tests, however, there were differences between strains and these are given individually for the strains from pathological material and the N.C.T.C. strains in Appendix A and summarized in Table 3. More detailed figures for the correlation between biochemical and fermentative activities of strains of A. lignieresi isolated from pathological material are given in Table 6 (p.64).

The positive reactions obtained in the methylene blue reduction tests did not go to completion and only a green colour was obtained. Hydrolysis of urea occurred at varying periods ranging from 24 hours up to 12 days. Hydrogen sulphide production was also variable in time, 53.6 per cent. of the strains giving a reaction within 24 hours, and 43.6 per cent. doing so in periods ranging from 2-7 days.

TABLE 3

Biochemical reactions of A. lignieresi strains

| Test for | Strains from pathological material | | N.C.T.C. Strains | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| Catalase production | 12 | 208 | 0 | 5 |
| V.P. test | 108 | 112* | 3 | 2* |
| Methylene blue reduction | 210‡ | 10 | 5 | 0 |
| Hydrogen sulphide production | 214 | 6 | 4 | 1 |
| Urease production | 81 | 139 | 1 | 4 |

* including weak positive reactions
‡ reaction incomplete

(e) Starch-forming properties

The examination of strains of A. lignieresi for their ability to synthesise starch was stimulated by the results obtained in preliminary experiments to demonstrate the breakdown of this substrate by these organisms. The organisms had been grown on 1 per cent. starch agar plates for 24 hours and each plate then flooded with Gram's iodine solution diluted 1 in 10. The medium became bluish-black but the bacterial

colonies themselves remained colourless. When the colonies were scraped off and more iodine solution added, however, the medium which had been covered by the colony became more intensely coloured than the surrounding area, suggesting that there had been some concentration of starch in the vicinity of the bacterial growth.

When strains were grown on dextrose-agar and treated with diluted Gram's iodine, some were found to give a bluish-purple or even black coloration of the bacterial growth itself (strong positive), whilst others showed a reddish-brown coloration of the organisms (weak positive). In no case was there any colour change of the bacterial growth when diluted Gram's iodine was added to cultures grown on nutrient agar slopes, showing that the reddish-brown colour seen in the weak positive reactions was not due to staining of the bacterial cytoplasm by the iodine.

Twenty strains of A. lignieresi were then tested for ability to produce starch on media containing various fermentable carbohydrates, some of which gave positive and some negative results in the fermentation tests. The results of these tests are set out in Table 4, from which it can be seen that positive

results were obtained most frequently on media containing dextrose and maltose and that strong positive reactions occurred more often on maltose agar than on dextrose agar.

Accordingly all strains were tested on dextrose and maltose agar and the results with these are summarized in Table 5. The reactions of individual strains are given in Appendix A and the correlation between the results with these tests and the other biochemical and fermentative tests is shown in Table 6 (p. 64).

TABLE 4

**Starch forming properties of 20 strains of A. lignieresi on medium containing various fermentable substrates**

| Strain No. | Nutrient-agar containing 1 per cent. of:- | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dextrose | maltose | laevulose | galactose | xylose | sucrose | lactose | dextrin | mannitol | dulcitol | salicin | inositol |
| A2 | ± | + | - | - | ± | ± | - | - | - | - | - | - |
| A3 | ± | + | - | - | ± | ± | - | - | - | - | - | - |
| A7 | + | + | ± | - | ± | ± | - | - | - | - | - | - |
| A12 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A20 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A30 | ± | + | - | - | ± | ± | - | - | - | - | - | - |
| A31 | ± | + | - | - | - | ± | - | - | - | - | - | - |
| A41 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A43 | - | - | - | - | - | - | - | - | - | - | - | - |
| A50 | ± | + | - | - | - | ± | - | - | - | - | - | - |
| A63 | ± | + | ± | - | - | - | - | - | - | - | - | - |
| A67 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A73 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A105 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A116 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A133 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A137 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A179 | ± | + | - | - | - | - | - | - | - | - | - | - |
| A191 | - | - | - | - | - | - | - | - | - | - | - | - |
| A199 | - | + | - | - | - | - | - | - | - | - | - | - |

+ = strong positive

± = weak positive

- = negative

TABLE 5

Starch-forming properties of *A. lignieresi* with two substrates

| No. of strains giving, with *dextrose* as substrate, test for starch formation | No. of strains giving, with *maltose* as substrate, test for starch formation | | | Totals for strains utilising *dextrose* to synthesise starch |
|---|---|---|---|---|
| | strongly positive | weakly positive | negative | |
| Strongly positive | 64 | 3 | 4 | 71 |
| Weakly positive | 95 | 26 | 7 | 128 |
| Negative | 13 | 4 | 9 | 26 |
| Totals for strains utilising *maltose* to synthesise starch | 172 | 33 | 20 | ... |

## 4. DISCUSSION

### (a) Primary cultures

The difficulty encountered, on occasions, in obtaining cultures of the organism from lesions of actinobacillosis, particularly in cattle, was noted by Lignières and Spitz (1902) who found it necessary, in some cases, to grind the pus in a sterile mortar before inoculation on to agar.

### (b) Low viability of strains

The low viability of A. lignieresi in culture has been observed by previous workers and appears to be quite characteristic of the organisms. Lignières and Spitz (1902) commented on the difficulty of maintaining cultures at incubator and room temperatures. They were of the opinion that "l'actinobacille est un microbe fragile ....". Griffith (1916) remarked upon the rapid diminution of the number of organisms in emulsions of tissues kept in the ice-chest and considered that serial subcultures must be made every 4 or 5 days to preserve the organism, but, using inspissated egg medium, he showed that cultures would remain alive up to 2 months. The tendency of A. lignieresi to die out quickly was noted by Bosworth

(1923) and by Cavandoli (1945) who regarded this as a characteristic of the organism. More recently, Till and Palmer (1960), in experiments to determine the survival time of A. lignieresi away from the animal body, found that it never exceeded 5 days on hay and straw. The ease with which the organism dies out would lend support to the idea of A. lignieresi occurring as a commensal organism rather than having a saprophytic existence.

(c) Host and tissue sources

The host-tissue distribution of strains of A. lignieresi found in this work is in accord with previous workers. Of the ovine strains, 95.83 per cent. were derived from abscesses about the head or in the lungs, which are the sites of lesions most commonly found (Magnusson, 1929; Jowett, 1931; Taylor, 1944), but the recovery of the organism from a brain abscess does not appear to have been recorded previously. The commonest site of lesions in cattle was found to be the tongue (88.78 per cent.) which is in agreement with other workers (Cavandoli, 1945; Till and Palmer, 1960). Lesions of the skeletal muscles in cattle, whilst not being common, have been described involving the lumbar

and thoracic muscles (Mawditt and Greenham, 1962). Actinobacillosis of the bony structures of the head has been described by Bosworth (1923) who isolated the organism from 8 such lesions in cattle. Similarly, cases involving the liver, although of infrequent occurrence, have been described by Davies and Torrance (1930) and by Till and Palmer (1960).

(d) Morphological characters

It is generally agreed that the usual morphology of A. lignieresi consists of short bacillary or even coccobacillary forms. Lignières and Spitz (1902) described such elements, but mentioned the development of streptobacillary forms in serum broth cultures. Subsequent workers have indicated that the organism may exhibit pleomorphism (Griffith, 1916; Davies and Torrance, 1930; Hayston, 1948; Till and Palmer, 1960). Griffith (1916) remarked upon the presence of occasional long forms in nutrient agar cultures, and Till and Palmer (1960) observed filamentous forms in broth cultures, the numbers of which increased up to 48 hours and then declined until, at 5 days, only the coccobacillary form of the organism was to be seen. Griffith (1916) also observed filamentous forms of

A. lignieresi when it was grown in dextrose-agar shake tubes, the colonies being "composed of a mass of long, tangled, unbranched filaments, not Gram-staining, with a variable number of smaller curved bacilli and circular bodies.". No other worker has recorded the association of the longer forms of the organism with the presence of dextrose or maltose in the medium.

The granules observed in cultures of A. lignieresi appear to be characteristic of this organism, although their existence has not previously been recorded. They may be the same structures as the "circular bodies" mentioned by Griffith (1916) but these were described only in dextrose-agar shake cultures and, since no illustration of them was given, cannot be compared with the present structures. The granules observed in the present work occurred on nutrient agar as well as on media containing dextrose and maltose. The absence of these granules in other Gram-negative bacteria makes this a valuable feature for the preliminary screening of organisms in the search for the commensal form of A. lignieresi.

The streptobacillary forms of A. lignieresi described by Lignières and Spitz (1902) have not been observed in the present work. It is unlikely that

these are the same as the organisms having the "streptococcal appearance" (Figs. 9 and 10) which were observed in the present strains, since the drawing of the streptobacillary forms published by Lignières and Spitz shows great regularity in the structures, whereas the filaments in the present work, which appear to have broken down, show considerable irregularity in the size and shape of the constituent elements.

Capsules have not been observed in A. lignieresi by other workers, but Arseculeratne (1962), describing a new species of actinobacillus (A. capsulatus) causing actinobacillosis in the joints of rabbits, demonstrated a well-marked capsule by means of the wet-film India-ink method used in the present work. In a preliminary report on this organism, Arseculeratne (1961) advanced the suggestion that the capsular material produced by the organisms in the lesions was responsible, in part, for the development of the "clubs" or "rays" typical of the lesion of actinobacillosis.

(e) Cultural characters

The adherence of colonies of A. lignieresi to the underlying medium has been described by Lignières and

Spitz (1902), Griffith (1916) and Ravaglia (1934). Lignieres and Spitz and Griffith observed that the property was lost on subculture, but that the colony remained viscous. Ristic et al. (1956) described three colonial forms of A. lignieresi, a granular form in which the colony exhibited adherence to the medium, and dwarf and fluorescent forms showing no adherence. Variations of this type have not been observed in the present work. Taylor (1944) found a variability in the degree of adhesion to the medium with his strains, and Till and Palmer (1960) found only 1 of their 26 strains which showed this feature. These latter workers discovered, however, that the use of reconstituted dehydrated media was attended by a number of strains showing adhesion to the medium and they concluded that adherence to the culture medium was dependent on environmental conditions and was not a consistent characteristic of the organism.

(f) Fermentation reactions

Several workers have studied the fermentative and biochemical properties of A. lignieresi in varying degrees, and their results are summarized in Table 7 (p. 65). Although Tunnicliff (1941) recorded

variable results with dextrose, all other workers obtained fermentation without gas formation. Similarly, there is almost complete agreement on the fermentation of maltose, mannitol, sucrose, xylose, laevulose, mannose and galactose, although maltose and laevulose were not fermented by the single strain examined by Mawditt and Greenham (1962), nor mannitol and xylose by the single strain of Sforza (1941). The late fermentation of sucrose seen with 3 of the strains of the present series was observed also by Ravaglia (1934) with his strain, and the late fermentation of galactose, seen with 44.1 per cent. of the present strains, was seen also by Till and Palmer (1960) whose strains fermented this sugar within 72 hours. Late fermentation of substrates, which, with the present series, gave prompt fermentation, has been recorded for maltose and mannitol (Till and Palmer, 1960) and xylose (Johnston, 1954; Till and Palmer, 1960). Fewer workers have examined the activity of A. lignieresi against dextrin, but Wramby (1940) and Ristic et al. (1956) reported variable results with this substrate, whilst Tunnicliff (1941) and Mawditt and Greenham (1962) obtained consistently negative results. In the present investigation all strains

fermented dextrin, a fact also observed by Taylor (1944), Till and Palmer (1960) and Vallée et al. (1963). With raffinose, discrepancies in the results by different workers are seen, Wramby (1940) and Taylor (1944) having obtained variable results, Vawter (1933) and Till and Palmer (1960) consistently positive reactions, and Magnusson (1929) and Sforza (1941) negative results. These observations are in accordance with the present findings where some 22 per cent. of strains fermented raffinose.

The non-fermentation of trehalose, inulin, dulcitol and inositol has been noted by all those who recorded the use of these substrates, with the exception of Till and Palmer (1960), who obtained fermentation of all four after 21 days incubation, and Vallée et al. (1963), who detected fermentation of inulin. Similarly, negative results were obtained with salicin in the hands of most workers, which is consistent with the present findings, although Wramby (1940) obtained variable results. Although rhamnose was not fermented by the strains examined by the majority of previous workers, the present finding of 1 strain which broke down this pentose, is matched by Mawditt and Greenham's strain (1962), whereas Till and

Palmer (1960) found late (21 days) fermentation of rhamnose by all their strains.

Only five previous workers have used glycerol, Wramby (1940), Tunnicliff (1941) and Taylor (1944) all obtaining variable results, whilst Vawter (1933) and Till and Palmer (1960) recorded late fermentation. In view of these results it is not surprising that the present work should have revealed 72.7 per cent. of strains giving late fermentation of glycerol. Arabinose has given divergent results in the past, Magnusson (1929), Vawter (1933), Tunnicliff (1941) and Pathak and Ristic (1962) having all obtained non-fermentation, whilst others have described positive results. This is in agreement with the present result when the majority of strains fermented arabinose after several days, but some 12 per cent. of the sample failed to do so. The late fermentation of lactose by A. lignieresi was considered by Taylor (1944) to be characteristic of the organism and has been reported by several authors (Magnusson, 1929; Jowett, 1931; Vawter, 1933; Wilson and Miles, 1955, p. 472). This has not proved to be a constant feature in this work, since 15.9 per cent. of strains were found not to ferment lactose within 14 days.

This, however, is not inconsistent with other results recorded in the literature, since Ravaglia (1934), Hayston (1948), Mawditt and Greenham (1962), Pathak and Ristic (1962) and Vallée *et al*. (1963) all mentioned the occurrence of non-lactose-fermenting strains.

Lack of activity of *A. lignieresi* strains against sorbitol was reported by all those who have recorded the use of this substrate, with the exception of Till and Palmer (1960) who found that it was fermented very slowly. The results in the present series support the findings of the majority of other workers, except for the existence of 3 strains (1.36 per cent.) which fermented sorbitol. The discrepant results obtained latterly with sorbitol are disturbing, especially as the supplies of "sorbitol" were quite obviously not uniform. The explanation for this non-uniformity was not apparent from the information given by the supplier. Sorbitol has been obtained by extraction from the berries of the mountain ash (*Sorbus aucuparia*) (Beilstein, 1918), but it is now synthesised by the hydrogenation of dextrose under pressure (Rule, 1943, p. 253; Cruickshank *et al*., 1960, p. 201). In the absence of any accurate source history for the samples of sorbitol used, one might suppose that the

more recent supplies of this alcohol may have contained traces of dextrose which would have been fermented by the actinobacillus strains, whereas the older sample, which may have been obtained by extraction from mountain ash berries, did not contain such impurities. It is interesting to note that all the workers who reported non-fermentation of sorbitol by their strains of A. lignieresi, published their work in the period 1929-1944.

(g) Biochemical characters

The failure of the present strains of A. lignieresi to produce indole confirmed the findings of most other workers, although Lignières and Spitz (1902), when first describing the organism, recorded that it produced indole in small amounts, and this finding was repeated by Thompson (1933a), Johnston (1954) and Wilson and Miles (1955, p. 472). Growth on MacConkey's medium was recorded by Hayston (1948), Mawditt and Greenham (1962) and Till and Palmer (1960) who noted that the growth on this medium was feeble.

Reduction of nitrate to nitrite has been tested for previously by very few workers. Tunnicliff (1941) obtained positive results with this test, as also did

Ristic et al. (1956), Mawditt and Greenham (1962) and Vallée et al. (1963), but Till and Palmer (1960) had negative results with their strains of A. lignieresi. Positive results with the catalase test were given by all the strains examined by Ristic et al. (1956) and Till and Palmer (1960), but this is not in agreement with the present series of tests in which 94.55 per cent. of strains were catalase negative. Pathak and Ristic (1962), however, recorded variable weak or negative results with this test, although the two strains of bovine origin which they examined gave completely negative results.

Production of hydrogen sulphide appears to be a property possessed by the majority of the strains of A. lignieresi examined, and this is borne out by the results of other workers, Tunnicliff (1941), Till and Palmer (1960), Mawditt and Greenham (1962) and Pathak and Ristic (1962) having reported positive tests. However, negative tests for hydrogen sulphide were given by the strains examined by Magnusson (1929) and Ristic et al. (1956) and by some of the strains of Pathak and Ristic (1962). Few investigators have tested for urease activity in A. lignieresi, but those who have (Ristic et al., 1956; Mawditt and Greenham,

1962; Vallée et al., 1963) were able to demonstrate hydrolysis of urea.

The finding of negative methyl red tests in the present work is in agreement with the results of Till and Palmer (1960) and Mawditt and Greenham (1962). The Vöges-Proskauer test proved to be positive in every case with Till and Palmer's strains, but Mawditt and Greenham (1962) obtained a negative V.P. test with their strain. The reduction of methylene blue test has been carried out previously with A. lignieresi strains only by Till and Palmer (1960) and they reported a positive result in every case.

(h) Starch-forming properties

The production of starch by A. lignieresi has not been recorded previously but it is of interest since, if the organism exists in the commensal state, it may well be that it goes to make up the normal iodophilic flora of the rumen.

TABLE 6. FERMENTATION AND BIOCHEMICAL REACTIONS OF 220 STRAINS OF ACTINOBACILLUS LIGNIERESI ISOLATED FROM PATHOLOGICAL MATERIAL

| | Lactose late positive | Lactose negative | Sucrose positive | Sucrose late positive | Sorbitol positive | Sorbitol late positive | Sorbitol negative | Raffinose positive | Raffinose late positive | Raffinose negative | Arabinose positive | Arabinose late positive | Arabinose negative | Glycerol positive | Glycerol late positive | Glycerol negative | Galactose positive | Galactose late positive | Rhamnose positive | Rhamnose negative | Catalase positive | Catalase negative | V.P. test positive | V.P. test weak positive | V.P. test negative | Methylene blue redn positive | Methylene blue redn negative | Urease positive | Urease negative | $H_2S$ positive | $H_2S$ late positive | $H_2S$ negative | Starch from dextrose: positive | Starch from dextrose: weak positive | Starch from dextrose: negative | Starch from maltose: positive | Starch from maltose: weak positive | Starch from maltose: negative | Host: bovine | Host: ovine | Site of lesion: tongue | Site of lesion: lung | Site of lesion: rumen/reticulum | Site of lesion: other tissue |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactose late positive | 185 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lactose negative | | 35 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sucrose positive | 182 | 35 | 217 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sucrose late positive | 3 | 0 | | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sorbitol positive | 1 | 0 | 1 | 0 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sorbitol late positive | 2 | 0 | 2 | 0 | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sorbitol negative | 182 | 35 | 214 | 3 | | | 217 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose positive | 4 | 1 | 5 | 0 | 0 | 0 | 5 | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose late positive | 35 | 9 | 44 | 0 | 1 | 1 | 42 | | 44 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose negative | 146 | 25 | 168 | 3 | 0 | 1 | 170 | | | 171 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose positive | 3 | 1 | 4 | 0 | 0 | 0 | 4 | 1 | 1 | 2 | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose late positive | 161 | 28 | 187 | 2 | 1 | 2 | 186 | 4 | 41 | 144 | | 189 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose negative | 21 | 6 | 26 | 1 | 0 | 0 | 27 | 0 | 2 | 25 | | | 27 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol positive | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 2 | 1 | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol late positive | 139 | 21 | 157 | 3 | 1 | 2 | 157 | 4 | 31 | 125 | 2 | 139 | 19 | | 160 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol negative | 43 | 14 | 57 | 0 | 0 | 0 | 57 | 1 | 12 | 44 | 2 | 48 | 7 | | | 57 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose positive | 107 | 16 | 121 | 2 | 1 | 1 | 121 | 3 | 28 | 92 | 3 | 98 | 22 | 2 | 97 | 24 | 123 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose late positive | 78 | 19 | 96 | 1 | 0 | 1 | 96 | 2 | 16 | 69 | 1 | 91 | 5 | 1 | 63 | 33 | | 97 | | | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose positive | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose negative | 184 | 35 | 216 | 3 | 1 | 2 | 216 | 5 | 44 | 170 | 4 | 188 | 27 | 3 | 159 | 57 | 123 | 96 | | 219 | | | | | | | | | | | | | | | | | | | | | | |
| Catalase positive | 8 | 4 | 12 | 0 | 0 | 1 | 11 | 0 | 2 | 10 | 0 | 11 | 1 | 0 | 7 | 5 | 1 | 11 | 0 | 12 | 12 | | | | | | | | | | | | | | | | | | | | |
| Catalase negative | 177 | 31 | 205 | 3 | 1 | 1 | 206 | 5 | 42 | 161 | 4 | 178 | 26 | 3 | 153 | 52 | 122 | 86 | 1 | 207 | | 208 | | | | | | | | | | | | | | | | | | | |
| V.P. test positive | 93 | 15 | 108 | 0 | 0 | 1 | 107 | 3 | 21 | 84 | 3 | 93 | 12 | 1 | 87 | 20 | 66 | 42 | 1 | 107 | 3 | 105 | 108 | | | | | | | | | | | | | | | | | |
| V.P. test weak positive | 56 | 14 | 69 | 1 | 1 | 1 | 68 | 1 | 16 | 53 | 0 | 59 | 11 | 2 | 44 | 24 | 35 | 35 | 0 | 70 | 8 | 62 | | 70 | | | | | | | | | | | | | | | | |
| V.P. test negative | 36 | 6 | 40 | 2 | 0 | 0 | 42 | 1 | 7 | 34 | 1 | 37 | 4 | 0 | 29 | 13 | 22 | 20 | 0 | 42 | 1 | 41 | | | 42 | | | | | | | | | | | | | | | |
| Methylene blue redn positive | 176 | 34 | 207 | 3 | 0 | 2 | 208 | 5 | 41 | 164 | 4 | 180 | 26 | 3 | 153 | 54 | 117 | 93 | 1 | 209 | 12 | 198 | 104 | 66 | 40 | 210 | | | | | | | | | | | | | | |
| Methylene blue redn negative | 9 | 1 | 10 | 0 | 1 | 0 | 9 | 0 | 3 | 7 | 0 | 9 | 1 | 0 | 7 | 3 | 6 | 4 | 0 | 10 | 0 | 10 | 4 | 4 | 2 | | 10 | | | | | | | | | | | | | |
| Urease positive | 67 | 14 | 81 | 0 | 0 | 1 | 80 | 0 | 12 | 69 | 1 | 65 | 15 | 1 | 63 | 17 | 49 | 32 | 0 | 81 | 1 | 80 | 42 | 24 | 15 | 76 | 5 | 81 | | | | | | | | | | | | |
| Urease negative | 118 | 21 | 136 | 3 | 1 | 1 | 137 | 5 | 32 | 102 | 3 | 124 | 12 | 2 | 97 | 40 | 74 | 65 | 1 | 138 | 11 | 128 | 66 | 46 | 27 | 134 | 5 | | 139 | | | | | | | | | | | |
| $H_2S$ positive | 98 | 20 | 115 | 3 | 0 | 1 | 117 | 3 | 21 | 94 | 2 | 103 | 13 | 1 | 82 | 35 | 65 | 53 | 1 | 117 | 10 | 108 | 49 | 44 | 25 | 112 | 6 | 36 | 82 | 118 | | | | | | | | | | |
| $H_2S$ late positive | 83 | 13 | 96 | 0 | 1 | 1 | 94 | 2 | 22 | 72 | 2 | 81 | 13 | 2 | 75 | 19 | 54 | 42 | 0 | 96 | 2 | 94 | 57 | 22 | 17 | 92 | 4 | 41 | 55 | | 96 | | | | | | | | | |
| $H_2S$ negative | 4 | 2 | 6 | 0 | 0 | 0 | 6 | 0 | 1 | 5 | 0 | 5 | 1 | 0 | 3 | 3 | 4 | 2 | 0 | 6 | 0 | 6 | 2 | 4 | 0 | 6 | 0 | 4 | 2 | | | 6 | | | | | | | | |
| Starch from dextrose: positive | 54 | 17 | 71 | 0 | 0 | 0 | 71 | 3 | 30 | 38 | 1 | 63 | 7 | 1 | 46 | 24 | 39 | 32 | 0 | 71 | 2 | 69 | 32 | 22 | 17 | 68 | 3 | 22 | 49 | 34 | 34 | 3 | 71 | | | | | | | |
| Starch from dextrose: weak positive | 108 | 15 | 121 | 2 | 1 | 0 | 122 | 2 | 12 | 109 | 2 | 107 | 14 | 2 | 96 | 25 | 68 | 55 | 1 | 122 | 9 | 114 | 60 | 42 | 21 | 116 | 7 | 47 | 76 | 72 | 49 | 2 | | 123 | | | | | | |
| Starch from dextrose: negative | 23 | 3 | 25 | 1 | 0 | 2 | 24 | 0 | 2 | 24 | 1 | 19 | 6 | 0 | 18 | 8 | 16 | 10 | 0 | 26 | 1 | 25 | 16 | 6 | 4 | 26 | 0 | 12 | 14 | 12 | 13 | 1 | | | 26 | | | | | |
| Starch from maltose: positive | 141 | 26 | 164 | 3 | 1 | 1 | 165 | 5 | 37 | 125 | 4 | 147 | 16 | 3 | 123 | 41 | 93 | 74 | 1 | 166 | 8 | 159 | 76 | 56 | 35 | 158 | 9 | 57 | 110 | 98 | 66 | 3 | 64 | 90 | 13 | 167 | | | | |
| Starch from maltose: weak positive | 30 | 3 | 33 | 0 | 0 | 0 | 33 | 0 | 2 | 31 | 0 | 26 | 7 | 0 | 21 | 12 | 18 | 15 | 0 | 33 | 3 | 30 | 19 | 10 | 4 | 32 | 1 | 17 | 16 | 10 | 20 | 3 | 3 | 26 | 4 | | 33 | | | |
| Starch from maltose: negative | 14 | 6 | 20 | 0 | 0 | 1 | 19 | 0 | 5 | 15 | 0 | 16 | 4 | 0 | 16 | 4 | 12 | 8 | 0 | 20 | 1 | 19 | 13 | 4 | 3 | 20 | 0 | 7 | 13 | 10 | 10 | 0 | 4 | 7 | 9 | | | 20 | | |
| Host: bovine | 162 | 34 | 193 | 3 | 1 | 2 | 193 | 4 | 35 | 157 | 4 | 167 | 25 | 2 | 142 | 52 | 109 | 87 | 1 | 195 | 11 | 185 | 93 | 63 | 40 | 188 | 8 | 70 | 126 | 107 | 86 | 3 | 54 | 117 | 25 | 145 | 31 | 20 | 196 | |
| Host: ovine | 23 | 1 | 24 | 0 | 0 | 0 | 24 | 1 | 9 | 14 | 0 | 22 | 2 | 1 | 18 | 5 | 14 | 10 | 0 | 24 | 1 | 23 | 15 | 7 | 2 | 22 | 2 | 11 | 13 | 11 | 10 | 3 | 17 | 6 | 1 | 22 | 2 | 0 | | 24 |
| Site of lesion: tongue | 148 | 26 | 171 | 3 | 1 | 1 | 172 | 4 | 30 | 140 | 4 | 146 | 24 | 2 | 126 | 46 | 99 | 75 | 1 | 173 | 8 | 166 | 85 | 54 | 35 | 167 | 7 | 63 | 111 | 92 | 79 | 3 | 49 | 103 | 22 | 130 | 30 | 14 | 174 | 0 |
| Site of lesion: lung | 11 | 1 | 12 | 0 | 0 | 1 | 11 | 0 | 4 | 8 | 0 | 12 | 0 | 1 | 8 | 3 | 8 | 4 | 0 | 12 | 2 | 10 | 7 | 4 | 1 | 11 | 1 | 6 | 6 | 6 | 6 | 0 | 6 | 4 | 2 | 9 | 1 | 2 | 3 | 9 |
| Site of lesion: rumen/reticulum | 5 | 1 | 6 | 0 | 0 | 0 | 6 | 0 | 0 | 6 | 0 | 6 | 0 | 0 | 5 | 1 | 3 | 3 | 0 | 6 | 1 | 5 | 3 | 1 | 2 | 5 | 1 | 2 | 4 | 4 | 2 | 0 | 1 | 5 | 0 | 6 | 0 | 0 | 6 | 0 |
| Site of lesion: other tissue | 21 | 7 | 28 | 0 | 0 | 0 | 28 | 1 | 10 | 17 | 0 | 25 | 3 | 0 | 21 | 7 | 13 | 15 | 0 | 28 | 1 | 27 | 13 | 11 | 4 | 27 | 1 | 10 | 18 | 16 | 9 | 3 | 15 | 11 | 2 | 22 | 2 | 4 | 13 | 15 |

TABLE 7

Results of fermentation and biochemical tests by previous workers

| | Bosworth (1923) | Davis and Stiles (1939) | Davies and Torrance (1930) | Griffith (1916) | Hayston (1948) | Johnston (1954) | Jowett (1931) | Lignieres and Spitz (1902) | Magnusson (1929) | Mawditt and Greenham (1962) | Pathak and Ristic (1962) | Ravaglia (1934) | Ristic et al. (1956) | Sforza (1941) | Taylor (1944) | Thompson (1933a) | Till and Palmer (1960) | Tunnicliff (1941) | Vallée et al. (1963) | Vawter (1933) | Wilson and Miles (1955) | Wramby (1940) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of strains | 17 | 6 | 16 | 23 | 1 | 1 | several | ... | 6 | 1 | 8 | 1 | 14 | 1 | 10 | 15 | 26 | 7 | 5 | 17 | | 3 |
| Dextrose | + | + | + | + | + | $+_1$ | + | + | + | + | + | + | + | + | + | + | $+_1$ | $+_v$ | + | $+_r$ | $+_r$ | + |
| Laevulose | + | + | + | ... | ... | ... | ... | ... | + | - | ... | ... | $+_v$ | + | + | ... | $+_1$ | + | ... | $+_r$ | ... | + |
| Galactose | + | ... | + | ... | ... | ... | ... | ... | + | ... | ... | ... | + | ... | + | ... | $+_3$ | + | ... | $+_r$ | ... | + |
| Mannose | ... | ... | ... | ... | ... | ... | ... | ... | + | + | $+_v$ | ... | $+_v$ | + | + | ... | $+_1$ | + | ... | ... | ... | + |
| Arabinose | ... | ... | ... | ... | ... | $+_4$ | ... | ... | - | + | - | ... | $+_v$ | + | $+_v$ | ... | $+_3$ | - | ... | - | ... | $+_v$ |
| Xylose | ... | + | + | ... | ... | $+_4$ | ... | ... | + | + | ... | ... | $+_v$ | + | + | ... | $+_3$ | + | + | $+_v$ | ... | + |
| Rhamnose | ... | ... | ... | ... | ... | - | ... | ... | - | + | - | ... | ... | - | - | ... | $+_{21}$ | - | ... | ... | ... | - |
| Sucrose | + | + | + | ... | + | $+_1$ | + | ... | + | + | + | $+_1$ | $+_v$ | + | + | + | $+_1$ | + | + | $+_r$ | $+_r$ | + |
| Maltose | + | + | + | ... | ... | $+_1$ | + | ... | + | - | + | + | + | + | + | + | $+_3$ | + | + | $+_r$ | $+_r$ | + |
| Lactose | + | + | + | + | - | $+_3$ | $+_6$ | + | $+_1$ | - | $-_v$ | - | ... | + | $+_1$ | + | $+_3$ | + | - | $+_1$ | $+_1$ | $+_v$ |
| Trehalose | ... | ... | ... | ... | ... | ... | ... | ... | ... | - | - | ... | ... | ... | - | ... | $+_{21}$ | - | ... | ... | ... | - |
| Raffinose | ... | ... | ... | ... | ... | ... | ... | ... | - | ... | ... | ... | ... | - | $+_v$ | ... | $+_{21}$ | - | ... | $+_1$ | ... | $+_v$ |
| Inulin | ... | ... | - | ... | ... | ... | ... | ... | - | - | ... | ... | ... | - | - | - | $+_{21}$ | ... | + | - | ... | - |
| Dextrin | ... | ... | ... | ... | ... | ... | ... | ... | ... | - | ... | ... | $+_v$ | - | + | ... | $+_7$ | - | + | ... | ... | $+_v$ |
| Glycerol | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | $+_v$ | ... | $+_7$ | $+_v$ | ... | $+_1$ | ... | $+_v$ |
| Mannitol | ... | ... | + | ... | + | $+_1$ | + | ... | + | + | ... | + | ... | - | + | + | $+_3$ | + | ... | $+_r$ | $+_r$ | + |
| Dulcitol | ... | ... | - | ... | ... | ... | - | ... | - | - | - | ... | ... | - | ... | - | $+_{21}$ | ... | ... | - | ... | - |
| Sorbitol | ... | ... | - | ... | ... | ... | ... | ... | - | ... | ... | ... | ... | - | - | ... | $+_{21}$ | ... | ... | ... | ... | - |
| Salicin | ... | ... | - | ... | ... | ... | - | ... | - | - | - | ... | ... | ... | - | - | - | ... | ... | - | ... | $+_v$ |
| Inositol | ... | ... | - | ... | ... | ... | ... | ... | ... | - | - | ... | ... | - | - | ... | $+_{21}$ | ... | ... | ... | ... | - |
| Indole | - | ... | - | ... | ... | ± | ... | ± | - | - | - | ... | - | ... | - | ± | - | - | - | ... | ± | ... |
| Nitrate redn. | ... | ... | ... | ... | ... | ... | ... | ... | ... | + | ... | ... | + | ... | ... | ... | - | + | + | ... | ... | ... |
| $H_2S$ | ... | ... | ... | ... | ... | ... | ... | ... | - | + | $+_v$ | ... | - | ... | ... | ... | + | + | ... | ... | ... | ... |
| Urease | ... | ... | ... | ... | ... | ... | ... | ... | ... | + | ... | ... | + | ... | ... | ... | ... | ... | + | ... | ... | ... |
| Catalase | ... | ... | ... | ... | ... | ... | ... | ... | ... | + | $+_v$ | ... | + | ... | ... | ... | + | ... | ... | ... | ... | ... |

Sugar fermentation

+ = fermentation (time not specified)
$+_1$; $+_{21}$ = fermentation within 1 day; 21 days
$+_r$ = rapid fermentation
$+_l$ = late fermentation
$+_v$ = fermentation by some but not all strains
$-_v$ = no fermentation

Biochemical tests

+ = positive reaction
± = weak positive reaction
$+_v$ = positive reaction by some but not all strains
$-_v$ = negative reaction

... = no information given

## Part II: The antigenic structure of *Actinobacillus lignieresi* recovered from pathological material.

## 1. INTRODUCTION

In their original description of Actinobacillus lignieresi Lignières and Spitz (1902) mentioned the use of a serum agglutination test as a diagnostic measure for actinobacillosis of cattle, but, whilst remarking upon a variability in the speed of the reaction with sera from a number of experimental animals, they did not investigate the possible existence of antigenic types of this organism. This possibility has been considered by a number of workers since that time, but the absence of conclusive results prompted Wilson and Miles (1955, p. 472), in their description of the antigenic structure of A. lignieresi, to the comment "little known".

Agglutination of A. lignieresi by antisera has been investigated by several workers with variable results. Davies and Torrance (1930) found that their strains all agglutinated with one antiserum and concluded that all were identical. Thompson (1933a), however, commented that "agglutinations and absorptions of agglutinins indicate that there is variation among strains of Actinobacillus as to antigenic structure.". Till and Palmer (1960) considered, from their results with agglutination and precipitation tests and

absorptions of antisera, that an antiserum prepared against any one strain of Actinobacillus should agglutinate all other strains, but that there were at least two serological types with the majority of their strains falling into one of these. Tunnicliff (1941), comparing ovine with bovine strains drew attention to the difficulty of identifying A. lignieresi by serological means, whilst Taylor (1944), also with strains from both cattle and sheep, believed that considerable antigenic differences may exist among strains of the organism.

Antigenic interrelationships between strains of A. lignieresi of human and those of bovine origin have been observed using the complement-fixation reaction (Beaver and Thompson, 1933) and the agar-gel diffusion precipitation technique (Pathak and Ristic, 1962), and antigens in common with other bacteria, viz. Pfeifferella mallei and Pf. whitmori (Beaver and Thompson, 1933; Thompson, 1933b), Actinobacillus equuli (Bacterium viscosum equi) (Vallée et al., 1963) and Pasteurella septica and P. haemolytica (Mawditt and Greenham, 1962), have been described.

It is the purpose in this section of the work to examine the antigenic characters of 218 of the strains

of A. lignieresi described in Part I.

## 2. MATERIALS AND METHODS

### (a) Source of strains of A. lignieresi

The strains of A. lignieresi used in this section of the work were those whose morphological, cultural and biochemical properties have been described in Part I, with the exception of strains no. A25 and A194 which died out during subculture on laboratory media. The five National Collection of Type Cultures strains (no. 4189, 4191, 4975, 4976 and 4985) were included in the investigation.

### (b) Media

Nutrient agar (infusion broth) used in the preparation of antigens was prepared as described by Mackie and McCartney (1948, pp. 142 and 148). Dextrose agar for the demonstration of antigenic material associated with the development of extracellular slime was prepared by the addition of 0.1 per cent. dextrose to nutrient agar.

### (c) Preparation of antigens

#### (i) Bacterial suspensions for tube agglutination tests.

Bacterial suspensions for use as antigens in the tube agglutination test were obtained by growing the

organisms on the surface of nutrient-agar slopes in 120-ml. medical flat bottles inoculated with 5 ml. of an overnight broth culture. After 18-20 hours at 37°C the growth was washed off with the broth inoculum and steamed at 100°C for 2 hours. After separation of the bacterial cells by centrifugation they were resuspended in saline (0.85 per cent. sodium chloride) solution to a density corresponding to a scale reading of 1.5 on an EEL portable colorimeter (Evans Electroselenium Ltd.) using a green (OGR1) filter. This density was approximately that of Brown's opacity standard no. 1 (Burroughs Wellcome). Suspensions of all strains of organisms subjected to the tube agglutination test were prepared in this way and are referred to as "heated antigens".

Two other types of suspensions were used with a small number of strains. In the preparation of both these suspensions the heating for 2 hours was omitted and the cells, after centrifugal separation, were resuspended in either saline solution (living antigen) or in saline containing 0.2 per cent. formaldehyde (formolised antigen). The living antigen was used at once, but heated and formolised antigens were stored in the refrigerator and used over periods of several weeks.

(ii) Bacterial suspensions for slide agglutination tests

Heavy suspensions of all strains of A. lignieresi for use as antigens in the slide agglutination test were obtained by growing the organisms overnight on nutrient agar slopes in 120-ml. medical flat bottles, the growth from one slope being washed off with 1 ml. of saline solution and the suspensions steamed for 2 hours. This is referred to as "heated slide antigen". In some experiments the suspensions were not steamed (living slide antigen).

(iii) Bacterial suspensions for immunisation

Suspensions of A. lignieresi strains for use as immunising agents in the preparation of antisera were prepared in the same way as the heated antigens for the tube agglutination test, except that the final density was adjusted to Brown's opacity tube no. 8. This is referred to as "heat-killed vaccine". A formolised vaccine was also employed and consisted of a nutrient broth culture of the organism incubated for 20 hours at 37°C and killed by the addition of 0.04 per cent. formaldehyde.

(d) Preparation of antisera

Rabbits were used for the preparation of antisera

against the heated cells of 22 strains of A. lignieresi and 6 antisera were obtained from goats. The strains used as immunising agents were A1, A2, A3, A4, A5, A6, A7, A8, A13, A14, A15, A17, A19, A20, A22, A33, A43, A46, A47, A49, A53, A55, A56, A59, A60, A75, A100 and A133. Prior to inoculation, rabbits and goats were bled to test for natural antibodies against the immunising strains. Rabbits received 7 intravenous injections of heat-killed vaccine at intervals of 4 days, the first and second doses being 0.25 and 0.5 ml., respectively, and thereafter 1 ml. Injections were made into the marginal vein of the right ear, the left ear being reserved for bleeding by venepuncture. Test-bleedings were made 7-10 days after the last injection and, if the agglutinating titre was satisfactory, further bleedings were made from the ear vein or by heart puncture. If the titre proved to be unsatisfactory further injections were given, followed by another test-bleeding. Goats were immunised on the same schedule, but injections were made into the jugular vein and test-bleedings were made at the same site.

Nine additional antisera were prepared against the living cells of A. lignieresi strains A5, A6, A7, A15,

A19, A46, A47, A49 and A133. The dosage schedules were the same as for the antisera against heated cells, but formolised vaccine was used for the first five injections and these were followed by two injections of a 20-hour nutrient broth culture of the immunising strain in doses of 0.25 ml. and 0.5 ml. The agglutinating titre of the serum was measured 7-10 days after the last injection and, if satisfactory, further bleedings were made.

Antisera were separated from the blood clot by centrifugation and preserved with 0.5 per cent. phenol by the addition of 1 ml. of saline containing 5 per cent. phenol to each 10 ml. of serum.

Antisera used for slide agglutination tests were diluted with saline solution containing 0.5 per cent. phenol until they gave readily observable agglutination of the homologous antigen within 1 minute on a slide. Initially dilution of antisera for slide agglutination tests was carried out with the mercuric iodide-saline used for the preservation of Salmonella agglutinating sera (for slide tests). This method was abandoned when it was found that all strains of A. lignieresi undergo spontaneous agglutination in mercuric iodide-saline.

(e) Agglutination tests

(i) Slide agglutination tests

Slide agglutination tests were carried out on a glass plate mounted over a black background with oblique lighting so that clumping of the bacterial cells could be seen without difficulty. Slide antigen was deposited on the glass plate with a large inoculating loop, an equal volume of antiserum then being added and the whole mixed with the same loop.

(ii) Tube agglutination tests

Tube agglutination tests were done in 2 in. x $\frac{1}{4}$ in. (50 mm. x 6 mm.) test-tubes (Durham tubes) and incubated 18-20 hours in a waterbath at 56°C. Serial doubling dilutions of serum were made from 1 in 10 upwards, the range covered being determined by the titre of the serum for its homologous antigen. A spring-loaded syringe was used in the preparation of the serum dilutions and addition of agglutinable suspensions.

(f) Absorption of antisera

Absorbing strains of A. lignieresi were grown overnight on nutrient agar slopes in the same way as for the preparation of agglutinable suspensions. The

growth from 2 slopes was washed off with 2-3 ml. sterile saline solution. In some cases the suspension was then steamed for 2 hours. The bacterial cells (living or heated) were separated by centrifugation and resuspended in 2 ml. of a 1 in 5 dilution of the serum to be absorbed. After incubation at 37°C for 2 hours, the serum-antigen mixture was allowed to stand overnight in the refrigerator at 4°C and the cells then removed by centrifugation.

A single tube agglutination test with equal volumes of the absorbed serum and the tube antigen of the absorbing strain of A. lignieresi was then used to show that absorption was complete. If a positive result was obtained with this test, further absorption of the serum was carried out with the growth from two additional 120-ml. nutrient agar slopes. Finally, when all antibodies against the absorbing strain had been shown to have been removed, a tube agglutination test was set up with the absorbed serum against its homologous antigen, the serial dilutions being taken two steps beyond the known titre of the unabsorbed serum.

(g) Demonstration of extracellular antigenic material

Cultures of A. lignieresi grown on 0.1 per cent. dextrose-agar for 18-20 hours were washed off with saline solution and the suspension incubated at 37°C for 2 hours. The cells were deposited by centrifugation and the supernatant fluid examined in precipitation tests against A. lignieresi antisera prepared against living antigens. The precipitation tests were carried out in capillary tubes which were partially filled with serum followed by the saline extract, the tube then being supported in "Plasticine" for incubation at 37°C for 2 hours.

## 3. RESULTS

### (a) Slide agglutination tests

Heated slide antigens of the 28 strains of *A. lignieresi* against which antisera had been prepared were tested against each of the antisera. The results of these tests are shown in Table 8. The 28 organisms so examined could be arranged in 6 types on the basis of these results, all except two of the strains (A49 and A20) being grouped in this way. There appeared to be a degree of antigenic overlap between some of the types, this being shown most markedly with the three antisera against strains A47, A13 and A49. Strain A47, whilst giving an agglutination pattern similar to organisms of type 1, produced an antiserum which agglutinated organisms of types 2 (A3 and A5) and 4 (A7, A8, A33 and A53) in addition to those of type 1. In the same way strain A13, falling into the type 5 agglutination pattern, gave an antiserum reacting with organisms of types 2 (A3 and A5), 4 (A7, A8, A33 and A53) and 6 (A17). Although strain A49 was one of the two strains which it was not possible to type, its antiserum agglutinated organisms in types 2 (A3 and A5), 4 (A7, A8, A33 and A53), 5 (A13, A43, A59 and A75)

TABLE 8

Cross agglutination reactions between 28 strains of A. lignieresi and their antisera

| Serum Antigen | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| A2 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A4 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A19 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A22 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A46 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| A47 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A55 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A56 | + | + | + | + | + | + | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A60 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A100 | + | + | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A133 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A3 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | + | - | - | - | - | + | - | - | - | - | - | + | - |
| A5 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - | + | - |
| A6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| A7 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| A8 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| A33 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| A53 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| A13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| A43 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| A59 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| A75 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| A15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | + | - | - |
| A17 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | + | + | + | - |
| A49 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | + | - |
| A20 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + |

+ = agglutination

+ = homologous strain

- = no agglutination

and 6 (A17). To a lesser extent antiserum A53 showed cross agglutination with organisms of antigenic type 6 (A15 and A17).

Slide agglutination tests using heated slide antigens of all 218 strains of A. lignieresi included in this work were carried out with the 28 antisera. The results of these tests with individual organisms are given in Appendix C and the distribution of these strains between the 6 antigenic types is given in Table 9. Only 15 strains could not be placed into those types previously demonstrated and of these 4 underwent spontaneous agglutination in saline. The other 11 strains, although agglutinating with several of the antisera, did not correspond with the agglutination patterns of any of the types, but an interesting feature was that all these strains agglutinated with antiserum A4, as did strains A49 and A20 (see Table 8). Twenty-five organisms differed slightly from the strains which were placed in type 1 in not agglutinating with one or two of the 10 sera characterising the group and these have been designated subtype 1a strains. Similarly, 3 strains, which differed slightly from the agglutination pattern characterising type 4 organisms, have been listed as

TABLE 9

Distribution of slide agglutination patterns in 218 strains of A. lignieresi

| Type or Subtype | Antisera causing agglutination | No. of strains from | | Total no. of strains |
|---|---|---|---|---|
| | | Cattle | Sheep | |
| 1 | A1, A2, A4, A19, A22, A46, A47, A55, A56, A60 | 119 | 0 | 119 |
| 1a | As in Group 1 but 1 or 2 sera fail to agglutinate | 25 | 0 | 25 |
| 2 | A3, A5 | 5 | 8 | 13 |
| 3 | A6 | 0 | 6 | 6 |
| 4 | A7, A33, A53 | 0 | 7 | 7 |
| 4a | As in Group 4 but A8 or A33 fail to agglutinate | 2 | 1 | 3 |
| 5 | A13, A43, A75 | 21 | 0 | 21 |
| 6 | A15, A17 | 9 | 0 | 9 |
| Untyped | A4 but no others in common | 11 | 0 | 11 |
| Untyped | Not agglutinated | 2 | 2 | 4 |
| | Total | 194 | 24 | 218 |

subtype 4a strains.

Slide agglutination tests on the five National Collection of Type Cultures strains showed that they also fitted into the agglutination patterns shown by other strains. Strains NCTC 4975 and 4976 were of antigenic type 1, and the 3 other strains were of antigenic subtype 4a.

(b) Tube agglutination tests

Twenty-four antisera against the organisms which could be typed were tested against heated antigens of the other strains of the same antigenic type to ascertain the agglutinating titres. The results of these tests are shown in Tables 10 and 11. All antisera of antigenic type 1 agglutinated all strains in the type, even antisera A100 and A133 which had not agglutinated all strains in slide tests. Antisera in the other types also agglutinated all the organisms contained in the same type, except antiserum A14 which agglutinated antigen A6 only at a low level.

Antisera A13 and A47, which were found to give a more extensive pattern of cross agglutination than other antisera in the slide agglutination tests, were examined in tube agglutination tests against the heated antigens of those strains which had been found

TABLE 10

**Agglutination titres of 12 antisera before and after absorption with heated suspensions of the 12 A. lignieresi strains of antigenic type 1 used to produce the antisera**

| Antiserum against strain | | Agglutinable and absorbing suspensions prepared with strain | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 |
| A1 | a* | 160 | 160 | 80 | 160 | 80 | 160 | 80 | 160 | 160 | 80 | 80 | 160 |
| | b | | 10 | N | N | N | N | N | N | N | N | N | N |
| A2 | a | 80 | 80 | 80 | 80 | 40 | 80 | 40 | 80 | 80 | 40 | 80 | 80 |
| | b | N | | N | N | N | N | N | N | N | N | N | N |
| A4 | a | 40 | 40 | 80 | 80 | 40 | 40 | 40 | 40 | 40 | 40 | 80 | 80 |
| | b | N | N | | N | N | N | N | N | N | N | N | N |
| A19 | a | 80 | 40 | 80 | 80 | 40 | 80 | 40 | 40 | 80 | 80 | 80 | 80 |
| | b | N | N | N | | N | N | N | N | N | N | N | 10 |
| A22 | a | 2560 | 2560 | 2560 | 2560 | 2560 | 1280 | 2560 | 2560 | 5120 | 1280 | 2560 | 1280 |
| | b | N | N | N | N | | N | N | N | N | N | N | N |
| A46 | a | 1280 | 2560 | 1280 | 2560 | 1280 | 2560 | 1280 | 2560 | 2560 | 1280 | 1280 | 1280 |
| | b | 10 | N | N | N | N | | N | N | N | N | N | 20 |
| A47 | a | 80 | 80 | 160 | 160 | 80 | 160 | 160 | 80 | 160 | 80 | 40 | 80 |
| | b | N | N | N | N | N | N | | N | N | N | N | N |
| A55 | a | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 40 | 20 | 20 | 20 | 20 |
| | b | N | N | N | N | N | N | N | | N | N | N | N |
| A56 | a | 80 | 80 | 160 | 160 | 80 | 160 | 80 | 80 | 160 | 80 | 80 | 80 |
| | b | N | N | N | N | N | N | N | N | | N | N | N |
| A60 | a | 160 | 160 | 320 | 160 | 80 | 320 | 160 | 160 | 160 | 320 | 160 | 160 |
| | b | N | N | N | N | N | N | N | N | N | | N | 40 |
| A100 | a | 1280 | 640 | 1280 | 1280 | 640 | 1280 | 640 | 1280 | 1280 | 640 | 2560 | 1280 |
| | b | N | N | N | 20 | 10 | N | 10 | N | N | 10 | | 80 |
| A133 | a | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 | 160 |
| | b | N | N | N | N | N | N | N | N | N | N | N | |

*a = reciprocal titre before absorption with strain indicated.

b = reciprocal titre with homologous organism after absorption with strain indicated.

N = no agglutination at a dilution of 1 in 10.

TABLE 11

Agglutination titres of 12 antisera before and after absorption with heated suspensions of strains of *A. lignieresi* of antigenic types 2, 3, 4, 5 and 6

| Antiserum against strain | | Agglutinable and absorbing suspensions prepared with strain | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A3 | A5 | A6 | A14 | A7 | A33 | A53 | A13 | A43 | A75 | A15 | A17 |
| A3 | a* | 320 | 320 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | b | | N | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| A5 | a | 160 | 160 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | b | N | | | | | | | | | | | |
| A6 | a | ... | ... | 160 | 80 | ... | ... | ... | ... | ... | ... | ... | ... |
| | b | | | | 80 | | | | | | | | |
| A14 | a | ... | ... | 20 | 160 | ... | ... | ... | ... | ... | ... | ... | ... |
| | b | | | 80 | | | | | | | | | |
| A7 | a | ... | ... | ... | ... | 320 | 320 | 320 | ... | ... | ... | ... | ... |
| | b | | | | | | N | N | | | | | |
| A33 | a | ... | ... | ... | ... | 80 | 80 | 80 | ... | ... | ... | ... | ... |
| | b | | | | | N | | N | | | | | |
| A53 | a | ... | ... | ... | ... | 320 | 320 | 640 | ... | ... | ... | ... | ... |
| | b | | | | | 10 | N | | | | | | |
| A13 | a | ... | ... | ... | ... | ... | ... | ... | 2560 | 2560 | 2560 | ... | ... |
| | b | | | | | | | | | N | N | | |
| A43 | a | ... | ... | ... | ... | ... | ... | ... | 1280 | 1280 | 1280 | ... | ... |
| | b | | | | | | | | N | | N | | |
| A75 | a | ... | ... | ... | ... | ... | ... | ... | 320 | 320 | 320 | ... | ... |
| | b | | | | | | | | N | N | | | |
| A15 | a | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 160 | 160 |
| | b | | | | | | | | | | | | N |
| A17 | a | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 40 | 40 |
| | b | | | | | | | | | | | N | |

* See footnote to Table 10.

to be agglutinated. The results are shown in Table 12, from which it can be seen that, with most antigens, agglutination occurred only in low dilutions of antiserum.

TABLE 12

Agglutination reactions with antisera A13 and A47 before and after absorption with heated antigens of strains of A. lignieresi agglutinated in slide tests

| Anti-serum against strain | | Agglutinable and absorbing suspensions prepared with strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A3 | A5 | A7 | A13 | A17 | A20 | A33 | A47 | A49 | A53 |
| A13 | a* | 40 | 20 | 10 | 2560 | 10 | 80 | 10 | ... | 20 | 10 |
| | b | 2560 | 2560 | 2560 | | 2560 | 2560 | 2560 | ... | 2560 | 2560 |
| A47 | a | 80 | 40 | 10 | ... | ... | ... | 20 | 160 | ... | 20 |
| | b | 80 | 40 | 80 | ... | ... | ... | 80 | | ... | 80 |

*a = reciprocal titre with strain indicated

b = reciprocal titre with homologous organism after absorption with strain indicated

(c) Absorption tests

Each of the 24 antisera against the organisms which could be typed were absorbed with the organisms of the same antigenic type as their homologous organism. The results of the tests with these

absorbed sera against their homologous heated antigen are shown in Tables 10 and 11. With the exception of 10 of the 132 absorptions carried out with the type 1 strains, there was complete absorption of antibodies from these antisera and even when antibodies were still present they had been reduced to a low level, suggesting complete homogeneity of the 12 organisms tested. Similar results were obtained with absorptions done with antisera and antigens of the other antigenic types, except for type 3 in which the two representative strains, A6 and A14, whilst showing cross agglutination, differed from each other in not absorbing antibodies against the strain of organism homologous to the antiserum.

Antisera A13, A47 and A49 were absorbed with those strains of A. lignieresi with which they had cross-agglutinated in slide tests. The results with antisera A13 and A47 are shown in Table 12. Antiserum A13 was absorbed with 8 such strains and in every case, although antibodies against the absorbing strain were removed, the titre of the serum for the homologous organism remained at the same level as that of the unabsorbed serum. Three of the absorbed A13 antisera were further examined by slide agglutination against

the 8 absorbing strains and 3 type 5 strains (A13, A43 and A75). For comparison antiserum A13 absorbed with a type 5 strain (A75) was tested against the same 11 heated slide antigens, the results being shown in Table 13. From this it is clear that, although the 3 absorbing strains A5, A7 and A17 represent different antigenic types (2, 4 and 6 respectively), they removed the antibodies not only for organisms of their own type but also for those of the other two types and for strains A49 and A20 which were not typed. The 3 antisera obtained by these absorptions were specific for organisms of antigenic type 5. Strain A75, however, removed all antibodies from antiserum A13, including those giving cross agglutination with the 8 strains of different antigenic types.

With antiserum A47 absorbed with 5 cross-agglutinating strains (Table 12) complete absorption of the antibodies for these strains was also accomplished, but the titre for heated antigen A47 was unchanged. Similar results were obtained with antiserum A53 absorbed with strain A17, the titre for the homologous organism remaining unchanged even though antibodies against the absorbing strain had been completely removed.

TABLE 13

Agglutination reactions between antisera A13 absorbed with four strains of A. lignieresi and tested against 11 slide antigens

| Antiserum A13 absorbed with strain | Slide antigen | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A3 | A5 | A7 | A33 | A53 | A17 | A20 | A49 | A13 | A43 | A75 |
| A75 | - | - | - | - | - | - | - | - | - | - | - |
| A5 | - | - | - | - | - | - | - | - | + | + | + |
| A7 | - | - | - | - | - | - | - | - | + | + | + |
| A17 | - | - | - | - | - | - | - | - | + | + | + |

+ = agglutination

- = no agglutination

Antiserum A49 did not produce visible agglutination of the homologous heated antigen in tube agglutination tests, but the serum would agglutinate slide antigens. Absorption of this serum without prior dilution was performed with strains A3, A5, A7, A13, A17, A33, A43, A53 and A75, the absorbed serum then being tested by the slide agglutination method for the presence of antibodies against absorbing and homologous strains. The results with antiserum A49 differed from those with the other 2 antisera, since complete removal of antibodies for the homologous

strain was brought about with all the absorbing strains used.

The relationship of the 11 strains of A. lignieresi which could not be typed but were agglutinated by antiserum A4 (Table 9) was investigated by absorbing antiserum A4 with strain A49 and then testing it against slide antigens of the 11 strains (A20, A21, A24, A49, A61, A96, A101, A116, A138, A171 and A195). This absorption resulted in the removal of antibodies for all the untyped strains, but the serum still agglutinated heated antigen A4 to full titre and gave agglutination of slide antigens of a number of strains of antigenic type 1 (A47, A50, A95, 1/27/8, 2/10/6).

(d) Agglutination of living and formolised antigens

The characteristic viscous colonies observed with freshly isolated cultures of A. lignieresi suggested that some kind of capsular material might be present and, although attempts by microscopy to demonstrate capsules had proved unsuccessful (Part I, p.37 ), the evidence of the presence of extracellular slime prompted a further investigation into the existence of antigens associated with living but not killed bacterial cells.

Slide agglutination tests were carried out on living and heated suspensions of strains A1, A3 and A7 using two groups of antisera; antisera A1, A2, A3 and A33 had been prepared against heated bacterial cells and antisera A5, A6, A7 and A15 against living bacterial cells. The results obtained with this group of tests are shown in Table 14. With each bacterial strain tested, agglutination of the living organisms was obtained with the same three sera, viz. those prepared against the living bacterial cells, whilst the suspensions of heated cells gave patterns of agglutination which were quite distinct and corresponded to those of the three antigenic types represented by the strains tested, viz. types 1, 2 and 4.

Tube agglutination tests were used to test heated, formolised and living antigens of three A. lignieresi strains (A46, A47 and A49) against three pairs of antisera prepared with living and heated vaccines of the same three strains, the results being shown in Table 15. Strains A46 and A47 are both of antigenic type 1 but strain A49 is an organism which was not typed (Table 9). Antiserum A49 (living), whilst not agglutinating any heated antigens, did agglutinate

TABLE 14

Agglutination of suspensions of living and heated A. lignieresi cells by antisera against heated and living antigens

| Agglutinable suspension | | Antiserum prepared against | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | heated antigen | | | | living antigen | | | |
| Strain | Preparation | A1 | A2 | A3 | A33 | A5 | A6 | A7 | A15 |
| A1 | living | - | - | - | - | + | + | + | + |
| | heated | + | + | - | - | - | - | - | - |
| A3 | living | - | - | - | - | + | + | + | - |
| | heated | - | - | + | - | + | - | - | - |
| A7 | living | - | - | - | - | + | + | + | - |
| | heated | - | - | - | - | - | - | + | - |

+ = agglutination

- = no agglutination

TABLE 15

**Agglutination titres of 6 antisera prepared against living and heated suspensions of 3 strains of A. lignieresi and tested against heated, formolised and living suspensions of the immunising strains**

| Agglutinable suspension | | Antiserum prepared against | | | | | |
|---|---|---|---|---|---|---|---|
| | | heated organisms | | | living organisms | | |
| Strain | Preparation | A46 | A47 | A49 | A46 | A47 | A49 |
| A46 | heated | 1280 | 160 | N | 2560 | 5120 | N |
| | formolised | 1280 | 160 | N | 5120 | 2560 | 80 |
| | living | 10 | 10 | N | 640(1280) | 640 | 40(1280) |
| A47 | heated | 640 | 80 | N | 1280 | 2560 | N |
| | formolised | N | 10 | N | 640 | 1280 | 160 |
| | living | N | N | N | 640 | 640(640) | N (640) |
| A49 | heated | N | N | N | N | 10 | N |
| | formolised | N | N | N | 320 | 320 | 160 |
| | living | N | N | N | 10(1280) | 10(1280) | 40(1280) |

Titres are reciprocals of the highest dilution of antiserum giving agglutination of the test strain of actinobacillus. Figures in brackets are the reciprocal titres obtained when tests were incubated at 37°C instead of 56°C.

N = no agglutination at a dilution of 1 in 10.

formolised and living antigens of all three strains. Likewise, antisera A46 (living) and A47 (living) agglutinated formolised and living antigens A49 but not the heated antigen of the same organism. Antisera A46 (heated) and A47 (heated) agglutinated the heated antigens of the homologous organisms, but living antigens were unaffected. This failure to react with these two antisera was also observed with formolised antigen A47, but formolised antigen A46 was agglutinated like the heated antigens.

These results suggested that a heat-labile antigen may be present in living cells of actinobacilli. To test whether inactivation of the antigen occurred at the temperature at which the agglutination tests were incubated (56°C) several of the tests with living antigens and antisera against living organisms were repeated with incubation at 37°C. These results are also given in Table 15 which shows that, particularly with living antigen A49, incubation at the lower temperature gave a marked increase in the titre.

Absorption of antiserum A47 (living) with a living suspension of strain A49 resulted in the complete removal of antibodies for living antigens A47 and A49, although the titre for heated antigen A47 remained the

same. On the other hand, absorption of this antiserum with a heated suspension of strain A47 removed antibodies for the heated antigen A47 but left unchanged the titres against living antigens A47 and A49.

As evidence of extracellular slime produced by A. lignieresi had been found by microscopical methods, attempts were made to demonstrate its existence serologically by means of the precipitation test. Two antisera against living A. lignieresi suspensions (A19 and A133) were employed in these tests, the results being shown in Table 16. Although the two immunising strains are of the same antigenic type (1) the saline extract from strain A3 (antigenic type 2) gave a positive precipitation reaction with antiserum. Such an antigenic cross-relationship had not been observed using the heated antigens (Table 8). Furthermore, the extract from strain A133 was tested against the A133 antiserum prepared against a heated vaccine and no precipitation occurred.

The correlation between antigenic type and the biochemical activities of A. lignieresi strains was examined, the results being set out in Table 17. From this it can be seen that no gross differences in

the biochemical characters exist between the strains of the various antigenic types.

TABLE 16

Precipitation reactions between saline extracts of 6 strains of A. lignieresi and 2 antisera prepared against living suspensions

| Antiserum prepared against strain | Saline extracts prepared from strain | | | | | |
|---|---|---|---|---|---|---|
| | A3 | A15 | A6 | A7 | A19 | A133 |
| A133 | - | - | - | - | + | + |
| A19 | + | - | - | - | + | - |

+ = precipitation

- = no precipitation

TABLE 17

Fermentation and biochemical reactions of antigenic types of A. lignieresi

| | Antigenic type:- | | | | | | | Unt. 1* | Unt. 2* |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1a | 2 | 3 | 4 | 5 | 6 | | |
| Lactose late positive | 103 | 18 | 12 | 5 | 10 | 15 | 5 | 11 | 4 |
| Lactose negative | 16 | 7 | 1 | 1 | 0 | 6 | 4 | 0 | 0 |
| Sorbitol positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sorbitol late positive | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Sorbitol negative | 118 | 25 | 13 | 6 | 10 | 20 | 9 | 11 | 3 |
| Sucrose positive | 118 | 23 | 13 | 6 | 10 | 21 | 9 | 11 | 4 |
| Sucrose late positive | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Raffinose positive | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Raffinose late positive | 10 | 3 | 5 | 2 | 6 | 11 | 3 | 3 | 1 |
| Raffinose negative | 107 | 21 | 7 | 4 | 4 | 10 | 6 | 7 | 3 |
| Arabinose positive | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Arabinose late positive | 101 | 22 | 12 | 5 | 9 | 17 | 8 | 10 | 4 |
| Arabinose negative | 14 | 3 | 1 | 1 | 1 | 4 | 1 | 1 | 0 |
| Glycerol positive | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Glycerol late positive | 87 | 22 | 11 | 5 | 8 | 10 | 3 | 10 | 2 |
| Glycerol negative | 30 | 3 | 2 | 1 | 1 | 11 | 6 | 1 | 2 |
| Galactose positive | 63 | 12 | 7 | 3 | 9 | 17 | 7 | 3 | 0 |
| Galactose late positive | 56 | 13 | 6 | 3 | 1 | 4 | 2 | 8 | 4 |
| Rhamnose late positive | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhamnose negative | 118 | 25 | 13 | 6 | 10 | 21 | 9 | 11 | 4 |
| Catalase positive | 10 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Catalase negative | 109 | 25 | 12 | 6 | 10 | 21 | 9 | 11 | 3 |
| V.P. positive | 62 | 13 | 7 | 3 | 5 | 6 | 3 | 5 | 2 |
| V.P. weak positive | 37 | 4 | 5 | 3 | 2 | 9 | 4 | 4 | 2 |
| V.P. negative | 20 | 8 | 1 | 0 | 3 | 6 | 2 | 2 | 0 |
| Methylene blue redn. positive | 115 | 24 | 13 | 5 | 9 | 19 | 8 | 11 | 4 |
| Methylene blue redn. negative | 4 | 1 | 0 | 1 | 1 | 2 | 1 | 0 | 0 |
| $H_2S$ positive | 66 | 15 | 7 | 2 | 7 | 9 | 5 | 4 | 3 |
| $H_2S$ late positive | 52 | 10 | 6 | 2 | 3 | 10 | 4 | 7 | 0 |
| $H_2S$ negative | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 1 |
| Urease positive | 41 | 10 | 6 | 2 | 4 | 9 | 1 | 6 | 1 |
| Urease negative | 78 | 15 | 7 | 4 | 6 | 12 | 8 | 5 | 3 |
| Starch from dextrose positive | 15 | 10 | 8 | 4 | 7 | 14 | 8 | 3 | 2 |
| weak positive | 86 | 13 | 3 | 2 | 3 | 6 | 1 | 8 | 0 |
| negative | 18 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 2 |
| Starch from maltose positive | 87 | 18 | 11 | 5 | 9 | 17 | 8 | 8 | 3 |
| weak positive | 22 | 1 | 1 | 1 | 1 | 3 | 1 | 3 | 0 |
| negative | 10 | 6 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| Bovine | 119 | 25 | 5 | 0 | 2 | 21 | 9 | 11 | 2 |
| Ovine | 0 | 0 | 8 | 6 | 8 | 0 | 0 | 0 | 2 |
| Tongue | 109 | 19 | 5 | 0 | 2 | 19 | 7 | 10 | 1 |
| Lung | 1 | 0 | 4 | 0 | 4 | 0 | 1 | 0 | 2 |
| Rumen | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other tissues | 7 | 2 | 4 | 6 | 4 | 2 | 1 | 1 | 1 |
| TOTAL | 119 | 25 | 13 | 6 | 10 | 21 | 9 | 11 | 4 |

* Unt. 1 = organisms which agglutinated with some antisera
Unt. 2 = organisms which did not agglutinate with any antisera

## 4. DISCUSSION

Previous workers who have investigated the antigenic composition of A. lignieresi have employed agglutinable suspensions and antisera prepared in a number of ways, so that their results are not always strictly comparable. Davies and Torrance (1930), Jowett (1931) and Taylor (1944) used living vaccines to prepare their antisera, whilst Tunnicliff (1941) and Till and Palmer (1960) immunised with heat-killed suspensions and Thompson (1933a) with formalin-killed bacteria. Agglutinable suspensions consisting of living organisms (Taylor, 1944), heat-killed organisms (Till and Palmer, 1960) and phenol-killed bacteria (Magnusson, 1929; Tunnicliff, 1941) have been used to demonstrate antigen-antibody union. No one worker, however, has made a comparison of the different types of antigen or antisera.

The existence of a number of antigenic types of A. lignieresi has been suspected for a considerable time. Thompson (1933a), using 15 strains of the organism from cattle, showed that there was variation in the antigenic structure of actinobacilli but that cross-agglutination occurred with all strains. Tunnicliff (1941), most of whose strains were of ovine

origin, was able to show one main antigenic group, but other strains examined did not fit into this. Antigenic differences were also observed by Taylor (1944) who obtained cross-agglutination of 7 ovine strains with antisera prepared against these strains as well as with 2 antisera against bovine strains. He found with absorption tests that 5 of the ovine strains were antigenically homogeneous, but 2 which showed differences from the other sheep strains had antigens in common with bovine strains. In their investigation of 26 bovine strains, Till and Palmer (1960) came to the conclusion that at least 2 serological types of A. lignieresi exist, but they pointed out that 4 of the strains which they examined (including N.C.T.C. 4189) did not belong to either of these 2 types. Moreover, they found that the antiserum which they had prepared against strain N.C.T.C. 4189 was "strain specific", so that the evidence they presented would seem to point to the existence of at least 4 antigenic types.

Some evidence of the host specificity of the antigenic types has been shown in the present work, in the case of antigenic types 3 and 4. Although the number of ovine strains of A. lignieresi included in the collection is small, types 3 and 4 are represented

solely by organisms isolated from sheep. The most commonly occurring type in the organisms from bovine sources is type 1 which, together with subtype 1a, comprises 66.0 per cent. of the total sample and 74.2 per cent. of the bovine strains. Whilst the origin of the present collection of A. lignieresi strains is relatively restricted, being derived almost entirely from animals slaughtered at Edinburgh Abattoir, it is interesting to note that the National Collection of Type Cultures strains, all of which are of bovine origin (Cowan, 1958), fall into these antigenic types. The two type 1 strains (4975 and 4976) together with one of the subtype 4a strains (4985) were isolated in Britain while strains 4189 and 4191 were isolated by Thompson in America. Till and Palmer (1960) included the two strains N.C.T.C. 4975 and N.C.T.C. 4976 in their work and found that they fell into the larger of their serological groups, showing that 76.9 per cent. of the bovine strains examined by them were probably of antigenic type 1.

The clear division of actinobacilli into types on the basis of their heat-stable antigens suggests that the major antigens concerned with this are quite distinct. The results with antisera A13, A47, A49,

A53 and A4 provide evidence, however, that minor antigens exist which may produce cross-agglutination reactions.

The results with living and formolised suspensions have shown that these suspensions differ antigenically from those in which the bacteria have been killed by heat. This difference points to the existence of an envelope antigen which appears to be relatively non-specific, since cells with which it is associated are agglutinated by antisera against living organisms of unrelated heat-stable antigenic types. Such an envelope antigen may have been responsible for the considerable degree of cross-agglutination found by other workers. Davies and Torrance (1930), whilst not indicating their method of preparation of agglutinable suspensions, did use living organisms for the production of their single antiserum which agglutinated all 11 strains examined. This result may have been due to the non-specificity of an envelope antigen, but since their strains were all of bovine origin, it may have been due to complete homogeneity of the sample. Taylor (1944), however, obtained marked cross-agglutination between strains from cattle and sheep and their corresponding antisera, when he used living

organisms for antiserum production and for his agglutinable suspensions. The agglutinating titres in a number of cases were considerably lower than those for the homologous organisms. Jowett (1931) also found a number of strains which agglutinated with one antiserum at titres lower than that for the homologous strain.

No previous worker has used heat-killed suspensions of A. lignieresi of the type employed in this study, viz. heated at 100°C for 2 hours. Till and Palmer (1960) used a suspension of organisms which had been killed by exposure to 60°C for half an hour, and they were able to show marked cross-agglutination between strains, even those which were shown later by absorption tests to be antigenically distinct. It is notable, however, that with the precipitin test the degree of specificity which they obtained with the unabsorbed sera was the same as that with the agglutination test using absorbed sera. This result was probably linked with the fact that the acid-heat extraction method, used by Lancefield (1933) for the extraction of polysaccharide haptens from streptococcal cells, had been used in the preparation of the A. lignieresi antigens for the precipitin test.

Although no microscopic capsule can be demonstrated with A. lignieresi cells, the existence of extracellular slime may well be linked with a submicroscopic capsule which could bring about inagglutinability of the living cells tested with antisera against the more deep-seated heat-stable antigens. The results obtained with formolised antigen A46 tested against antisera A46 (heated) and A47 (heated) suggest that the envelope antigen may not always be so complete as to cause absolute inagglutinability of the cells. Variation in the amount of the envelope antigen might be expected to occur with repeated subcultivation, since the viscous character of the colonies of A. lignieresi may be lost after several subcultures.

## Part III: The commensal role of *Actinobacillus lignieresi*

## 1. INTRODUCTION

The habitat of Actinobacillus lignieresi, other than in lesions of actinobacillosis, is not known. It is generally accepted that, in actinobacillosis of cattle and sheep, the organism enters the tissues through wounds of the skin and of the oral and ruminal mucous membranes, and it has been suggested that it may exist as a commensal on the skin and in the mouth. The organism has not been isolated from such sites in normal animals, however, and these accepted views are based upon circumstantial evidence in outbreaks of the disease.

Most cases of actinobacillosis are sporadic in nature and usually there is no evidence of spread of infection from one animal to another. Lignières and Spitz (1902), however, encountered an epidemic of the disease which assumed alarming proportions. Sanders and Ristic (1956) investigated a series of cases occurring in a herd in which the disease had been unknown prior to the introduction, 5 months previously, of a bull affected with subcutaneous granulomatous lesions in the mandibular region.

In many cases where a number of animals are affected with the disease, it is believed that

managemental or environmental factors have played some part in the origin of the disease. Gerring (1947) reported three outbreaks of the disease in cattle in an area in which the practice of "burning-off" was employed prior to the sowing of grass seed in the ash, which was of a shaly nature. It was thought that ingestion of this ash may have caused damage to the epithelial tissues, thus allowing organisms to enter. Key and Loffer (1956) encountered a high incidence of actinobacillosis in a beef herd where, due to the breakdown of a hammermill, a coarse mixture was being fed temporarily, and Robinson (1951) and Hugo (1961) described similar predisposing factors associated with the feeding of "Balbala" and unchaffed bean hay, respectively. Infection of animals involved in an outbreak of an atypical form of actinobacillosis was believed by Hebeler et al. (1961) to be associated (a) with injuries to the mouth caused by a batch of poor quality hay and (b) with injuries to the skin produced by protruding bolt ends in an overcrowded pen.

In sheep, infection with A. lignieresi, associated with damage to the mucous membrane of the lips due to a number of agents in the food, has been reported. Christiansen (1917) suspected that spiny plants on

pastures may have caused damage to the tissues and allowed entry of the organisms. Thomas (1931), in a feeding experiment with prickly pear (Opuntia spp.), recorded a high incidence of actinobacillosis, and Davis and Stiles (1939) believed that infection had been introduced in their cases by the penetration of needle-grass awns through the skin. Damage by rice hulls in the diet may have been responsible for the high incidence of actinobacillosis in a group of young rams examined by Hayston (1948).

The route of entry of the infecting organisms has been deduced by some workers from the observed sites and distribution of the lesions. In the ovine cases examined by Taylor (1944), wounds appeared to be the portal of entry for the organisms, e.g. abrasions of the skin caused by wire fencing, damage to the pharynx by means of a balling-gun and bruising of the skin over the frontal bone in young rams as a result of fighting. Thornton (1943) drew attention to the numerous cases of actinobacillosis which had reached almost epidemic proportions on the Continent following attacks of foot-and-mouth disease, which he believed to indicate that damage to the oral mucous membrane was a probable mode of entry for the organisms. Davies and Torrance

(1930) were of the opinion that the common distribution of lesions in bovine actinobacillosis was such as to suggest that the channel of infection was injury to a mucous membrane. Even when organs with no mucosae were involved (e.g. liver, diaphragm, lung), they found that the oldest lesions were always in tissues and organs in which the mucous membrane was involved (e.g. tongue, hard palate and cheeks, reticulum, rumen and oesophagus).

The possible commensal nature of A. lignieresi has been hinted at by a number of workers. Magnusson (1929) stated that "It is more than probable that the bacillus .... belongs to the normal flora of the mouth ....". Till and Palmer (1960), investigating the hypotheses that A. lignieresi is either a soil organism or a commensal in the bovine mouth, concluded, because of the short survival time of the organism outwith the animal body, that the bovine mouth is more likely to be its natural habitat, although they were unable to demonstrate its presence there. Taylor (1944) was led to the conclusion that A. lignieresi may be a commensal of the mouth and/or skin of sheep.

The commensal nature of A. lignieresi remains conjecture, however, and confirmation of this role by

the isolation of the organism from normal cattle and sheep has not been made. The earlier work (Part I) with strains of A. lignieresi from pathological material and the demonstration of their starch-forming properties, coupled with the idea of A. lignieresi as part of the flora of the normal rumen, suggested that the examination of normal rumen contents might be useful. It is the purpose of this section of the work to describe the development of a selective medium suitable for the isolation of actinobacilli from a mixed microbial population and to report the isolation of such organisms from the rumen contents of both cattle and sheep and also from the normal bovine tongue. The fermentative and biochemical properties of these organisms are recorded and a superfical comparison of their antigens with those of A. lignieresi made.

## 2. MATERIALS AND METHODS

### (a) Development of a selective medium

The vast and varied flora of the mouth and rumen of cattle and sheep made it essential that a selective medium be devised to enable the search for actinobacilli in these situations to be carried out. Preliminary investigations of the type of colonies to be expected in cultures from the bovine mouth and rumen showed that a heavy growth of anthracoid organisms usually tended to blot out the growth of all the smaller colonial types; other organisms which predominated were the Gram-positive cocci.

The inhibitory effect of a number of substances on the growth of several strains of *A. lignieresi* was tested. The basic medium consisted of nutrient agar with the addition of 5 per cent. of lysed horse blood (lysed blood was used so that counting of bacterial colonies could be accomplished easily). Plates of media containing the inhibitory substances were inoculated with ten-fold dilutions of 20-hour nutrient broth cultures of the bacterial strains, using the dropping pipette technique of Miles and Misra (1938). Controls were set up at the same time on the basic medium. After incubation at 37°C for 20-24 hours,

the number of colonies was counted and, by comparison with the control plates, a measure of the inhibitory effect of the medium was obtained.

Initial tests with six strains of A. lignieresi showed that sodium azide, thallous acetate and brilliant green were completely inhibitory and their use was discontinued. The other agents and the concentrations in which they were tested are set out in Table 18 which shows the results with a further 11 strains of A. lignieresi together with other bacterial species. None of the agents tested inhibited the other bacteria whilst allowing A. lignieresi to grow without check, and the search along these lines was discontinued.

Antibiotic sensitivity tests were carried out using Evans "Sentests" on cultures of organisms grown on 5 per cent. horse blood agar plates. The organism to be tested was grown in nutrient broth overnight and then pipetted over the surface of the blood agar plate, the excess being removed and the plate then dried in the incubator for 30 minutes before the "Sentests" were placed in position. The antibiotics tested are shown in Table 19 (p. 112) in which the results with 57 strains of A. lignieresi and several other

TABLE 18

**11 strains of A. lignieresi tested on media containing inhibitory agents compared with 5 other species of bacteria**

| | A. lignieresi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of strains not inhibited | No. of strains partially inhibited | No. of strains totally inhibited | K. aerogenes | E. coli | Staph. aureus | Strep. faecalis | Bac. megatherium |
| Potassium Tellurite 1:50,000 | 6 | 4 | 1 | + | ± | + | - | - |
| Copper Sulphate 1:50,000 | 10 | 1 | 0 | + | + | + | - | - |
| 1:100,000 | 10 | 1 | 0 | + | + | + | - | ± |
| Teepol 1:1000 | 6 | 5 | 0 | + | + | + | - | - |
| 1:5000 | 8 | 3 | 0 | + | + | + | - | - |
| 1:10,000 | 9 | 2 | 0 | + | + | + | - | - |
| Crystal Violet 1:500,000 | 2 | 6 | 3 | + | + | - | - | - |
| 1:1,000,000 | 4 | 6 | 1 | + | + | - | - | - |
| Thionine 1:100,000 | 4 | 2 | 5 | + | + | ± | - | - |
| 1:500,000 | 7 | 4 | 0 | + | + | + | - | - |
| Basic Fuchsin 1:100,000 | 2 | 7 | 2 | + | + | - | - | - |
| 1:500,000 | 4 | 6 | 1 | + | + | - | ± | - |
| Methylene Blue 1:50,000 | 0 | 0 | 11 | + | + | - | - | - |
| 1:100,000 | 0 | 0 | 11 | + | + | - | ± | - |
| Sodium Taurocholate 1:200 | 2 | 7 | 1 | + | + | + | + | - |
| 1:500 | 5 | 6 | 1 | + | + | + | + | - |
| 1:1000 | 6 | 4 | 1 | + | + | + | + | - |
| Sodium Desoxycholate 1:2000 | 3 | 5 | 3 | + | + | + | ± | - |
| 1:5000 | 3 | 7 | 1 | + | + | + | ± | - |

\+ = growth equal to control

± = growth slightly inhibited

\- = no growth i.e. total inhibition

TABLE 19

Effect of antibiotics on the growth of A. lignieresi and other species of bacteria

| Antibiotic | Amount contained in each tablet | A. lignieresi | | | Klebsiella aerogenes | Escherichia coli | Staph. aureus | Streptococci (5 strains) | | | | | Anthracoid organisms (5 strains) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 42 strains | | 15 strains | | | | | | | | | | | | | |
| | | No. of strains sensitive | No. of strains resistant | No. of strains sensitive | No. of strains resistant | | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Penicillin | 0.5 units | 0 | 42 | 5 | 10 | R | R | R | S | R | R | S | S | S | R | R | R |
| Streptomycin | 20 µg. | 39 | 3 | 15 | 0 | R | S | S | S | R | R | S | S | S | S | S | S |
| Chloramphenicol | 40 µg. | 41 | 1 | 15 | 0 | S | S | S | S | S | S | S | S | S | S | S | S |
| Aureomycin | 10 µg. | 42 | 0 | 15 | 0 | S | R | S | S | R | R | S | S | S | S | S | S |
| Oxytetracycline | 10 µg. | 42 | 0 | 14 | 1 | S | R | S | S | R | R | S | S | S | S | S | S |
| Erythromycin | 1 µg. | ... | ... | 1 | 14 | R | R | R | S | S | S | S | S | S | S | S | S |
| Tetracycline | 10 µg. | ... | ... | 15 | 0 | S | S | S | S | ... | ... | ... | S | ... | ... | ... | ... |
| Neomycin | 10 µg. | ... | ... | 15 | 0 | S | S | S | S | S | S | R | S | S | S | S | S |
| Bacitracin | 100 µg. | ... | ... | 0 | 15 | R | R | R | S | S | S | R | R | R | R | R | R |
| Novobiocin | 2 µg. | ... | ... | 0 | 15 | R | R | R | S | S | S | R | S | S | S | S | S |
| Oleandomycin | 2 µg. | ... | ... | 0 | 15 | R | R | R | S | S | S | S | S | S | S | S | S |

bacterial species are given. Using a small range of antibiotics it was found that penicillin was the only agent which did not inhibit A. lignieresi, but a number of the other bacterial species were also resistant. When the number of antibiotics investigated was extended, A. lignieresi was found to be resistant to bacitracin, novobiocin and oleandomycin and of these, oleandomycin was the most inhibitory to the other bacterial species tested. Although Klebsiella aerogenes and Escherichia coli were resistant to oleandomycin, it was believed that these organisms would be less likely to give trouble in cultures prepared from the bovine mouth and rumen than would streptococci and anthracoid organisms which were sensitive to oleandomycin. Therefore, it was decided to use this antibiotic in the selective medium. The minimal inhibitory concentration (m.i.c.) of oleandomycin for A. lignieresi strains and other organisms was determined by the serial dilution tube technique (Cruickshank et al., 1960, p. 407). The m.i.c. for A. lignieresi was found to be 25 µg/ml., but the other organisms tested were inhibited by concentrations down to 0.75 µg/ml.

Preliminary experiments had shown that plates

inoculated with material from the bovine rumen frequently showed the presence of moulds, which often overgrew the other organisms present. It was decided, therefore, to incorporate a fungistatic agent in the selective medium, the one chosen being nystatin ("Mycostatin", Squibb).

The final selective medium consisted of nutrient agar containing 5 per cent. horse blood agar and 1 µg oleandomycin and 200 units nystatin per ml. This medium adequately supported the growth of pure cultures of A. lignieresi whilst inhibiting the growth of the streptococci, staphylococci and anthracoid organisms which had been used in the previous tests.

(b) Other media

The other media used for the isolation and examination of organisms included in this section of the work are those which were used in Part I for the examination of strains of A. lignieresi isolated from pathological material.

(c) Isolation of organisms from ruminal contents

Ruminal material was taken from normal cattle and sheep immediately after slaughter at the abattoir and inoculated, as soon as possible thereafter, on

antibiotic blood agar plates. Ruminal content samples were collected in polystyrene containers with snap-on lids which were sterilised after use by overnight soaking in sodium hypochlorite solution ("Chloros") followed by rinsing with distilled water.

After incubation of the antibiotic blood agar plates likely colonies were picked on to horse blood agar plates for further examination. The criteria for selection of colonies were:-

(1) a diameter of 1-2 mm.,

(2) a smooth, moist appearance,

(3) a viscous character apparent when the colony was taken up on the inoculating loop.

Smears were made from the subcultures and stained by Gram's method and those organisms having the morphology of A. lignieresi, in particular the granules and "Morse code" forms already described (p. 30), were examined in more detail biochemically and antigenically.

(d) Isolation of organisms from lingual swabs

Mucus from the lateral aspect of the tongues of normal cattle was taken, by means of swabs, immediately after slaughter and inoculated on antibiotic blood agar

plates. After incubation colonies were selected by the same criteria as used for the examination of ruminal samples.

(e) Biochemical reactions

Organisms isolated from the tongue and ruminal contents of normal cattle and sheep were subjected to the same group of biochemical tests (including tests for starch-production) as was used for the strains of A. lignieresi recovered from pathological material (p. 20).

(f) Preparation of antigens

A heated slide antigen of each strain isolated was prepared by the methods used in Part II (p. 70).

(g) Preparation of antisera

Antisera were prepared in rabbits against 2 strains of organisms isolated from the ruminal contents of cattle. These strains (43.4 and 97B3) were selected at random. The methods used were the same as those already described in Part II (p. 72).

(h) Absorption of antisera

Antisera were absorbed by the methods used in Part II (p. 75).

## 3. RESULTS

### (a) Incidence of actinobacillus-like organisms

#### (i) Cattle

Ruminal contents were taken from 306 cattle and lingual swabs from 105 animals. In some cases regurgitation of ruminal material at the time of slaughter had occurred, but such material was avoided in collecting mucus from the tongue. The two series of samples (rumen and tongue) were taken at different times so that samples of ruminal material and lingual mucus from the same animal are not included in the investigation.

As far as could be ascertained in the routine meat inspection carried out in the abattoir, none of the 411 animals sampled showed any macroscopic evidence of actinobacillosis. The sera of 189 of the animals from which ruminal material was taken were examined serologically for the presence of antibodies against various antigenic types of A. lignieresi and all gave values which fall within the range for normal cattle (see Part IV, p. 146 ).

Actinobacillus-like organisms were recovered from 31 (10.1 per cent.) of the samples of ruminal contents and from 39 (37.1 per cent.) of the lingual swabs.

(ii) Sheep

Ruminal contents were collected from 330 sheep. Actinobacillus-like organisms were isolated from 84 of these samples, giving an incidence of 25.5 per cent.

(b) Biochemical characters of the actinobacillus-like organisms

(i) Cattle

All 70 organisms isolated from the rumens and tongues of cattle fermented dextrose, laevulose, sucrose, maltose and mannitol promptly within 24 hours without the production of gas, but failed to ferment trehalose, inulin, dulcitol and salicin within 14 days. All strains grew on MacConkey's medium and reduced nitrates to nitrites. The methyl red test was negative with every strain as also were the tests for the production of indole, urease and ammonia. The methylene blue reduction test was weakly positive with every strain except one, a rumen strain, which brought about complete decolorisation of the dye. Hydrogen sulphide was produced by all strains, but the time at which this occurred varied between strains. Differences between strains were observed with the remaining fermentable substrates and biochemical tests

employed, and these are shown for the individual organisms in Appendix B and in a summarized form in Table 20. The inter-relationships between the various fermentative and biochemical activities of the organisms isolated from the rumens and tongues are given in Table 21 (p. 120) and Table 22 (p. 121) respectively.

TABLE 20

Biochemical reactions of actinobacilli from the bovine tongue and rumen

| Test | 31 rumen strains | | 39 lingual strains | |
|---|---|---|---|---|
| | No. of strains | | No. of strains | |
| | Positive | Negative | Positive | Negative |
| Fermentation of: | | | | |
| Mannose | 25 (20*) | 6 | 33 (22*) | 6 |
| Galactose | 30 (26*) | 1 | 37 (23*) | 2 |
| Arabinose | 31 (27*) | 0 | 36 (10*) | 3 |
| Rhamnose | 14 (11*) | 17 | 10 (7*) | 29 |
| Lactose | 29* | 2 | 35* | 4 |
| Raffinose | 23 (22*) | 8 | 29* | 10 |
| Glycerol | 27 (25*) | 4 | 36 (35*) | 3 |
| Inositol | 4* | 27 | 16* | 23 |
| Dextrin | 31 (12*) | 0 | 39 (18*) | 0 |
| Xylose | 31 (13*) | 0 | 39 (4*) | 0 |
| Catalase production | 28 | 3 | 36 | 3 |
| Vöges-Proskauer test | 30± | 1 | 30 (29±) | 9 |
| Starch synthesis from: | | | | |
| Dextrose | 11 (6±) | 20 | 14 (12±) | 25 |
| Maltose | 22 (10±) | 9 | 21 (10±) | 18 |

* Late fermentation (negative at 24 hr; positive within 14 days).
± Weakly positive.

TABLE 21. FERMENTATION AND BIOCHEMICAL REACTIONS OF 31 STRAINS OF ACTINOBACILLI ISOLATED FROM THE RUMINAL CONTENTS OF NORMAL CATTLE

| | Lactose late positive | Lactose negative | Raffinose positive | Raffinose late positive | Raffinose negative | Arabinose positive | Arabinose late positive | Arabinose negative | Glycerol positive | Glycerol late positive | Glycerol negative | Galactose positive | Galactose late positive | Galactose negative | Mannose positive | Mannose late positive | Mannose negative | Inositol late positive | Inositol negative | Rhamnose positive | Rhamnose late positive | Rhamnose negative | Sorbitol positive | Sorbitol late positive | Sorbitol negative | Dextrin positive | Dextrin late positive | Xylose positive | Xylose late positive | $H_2S$ positive | $H_2S$ late positive | Catalase positive | Catalase negative | V.P test weak positive | V.P test negative | Starch from dextrose: positive | Starch from dextrose: weak positive | Starch from dextrose: negative | Starch from maltose: positive | Starch from maltose: weak positive | Starch from maltose: negative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactose late positive | 29 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lactose negative | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose positive | 1 | 0 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose late positive | 21 | 1 | | 22 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose negative | 7 | 1 | | | 8 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose positive | 4 | 0 | 0 | 2 | 2 | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose late positive | 25 | 2 | 1 | 20 | 6 | | 27 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose negative | 0 | 0 | 0 | 0 | 0 | | | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol positive | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol late positive | 24 | 1 | 0 | 17 | 8 | 3 | 22 | 0 | | 25 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol negative | 3 | 1 | 0 | 4 | 0 | 1 | 3 | 0 | | | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose positive | 4 | 0 | 1 | 1 | 2 | 1 | 3 | 0 | 1 | 3 | 0 | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose late positive | 24 | 2 | 0 | 21 | 5 | 3 | 23 | 0 | 1 | 21 | 4 | | 26 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose negative | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose positive | 5 | 0 | 0 | 4 | 1 | 0 | 5 | 0 | 0 | 3 | 2 | 2 | 3 | 0 | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose late positive | 18 | 2 | 1 | 13 | 6 | 2 | 18 | 0 | 1 | 18 | 1 | 2 | 17 | 1 | | 20 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose negative | 6 | 0 | 0 | 5 | 1 | 2 | 4 | 0 | 1 | 4 | 1 | 0 | 6 | 0 | | | 6 | | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol late positive | 4 | 0 | 0 | 3 | 1 | 2 | 2 | 0 | 0 | 4 | 0 | 1 | 3 | 0 | 1 | 2 | 1 | 4 | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol negative | 25 | 2 | 1 | 19 | 7 | 2 | 25 | 0. | 2 | 21 | 4 | 3 | 23 | 1 | 4 | 18 | 5 | | 27 | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose positive | 3 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 3 | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose late positive | 10 | 1 | 0 | 10 | 1 | 0 | 11 | 0 | 1 | 10 | 0 | 1 | 10 | 0 | 1 | 8 | 2 | 0 | 11 | | 11 | | | | | | | | | | | | | | | | | | | | |
| Rhamnose negative | 16 | 1 | 0 | 10 | 7 | 4 | 13 | 0 | 0 | 14 | 3 | 2 | 14 | 1 | 3 | 10 | 4 | 4 | 13 | | | 17 | | | | | | | | | | | | | | | | | | | |
| Sorbitol positive | 21 | 1 | 0 | 17 | 5 | 4 | 18 | 0 | 1 | 17 | 4 | 2 | 19 | 1 | 4 | 14 | 4 | 3 | 19 | 2 | 8 | 12 | 22 | | | | | | | | | | | | | | | | | |
| Sorbitol late positive | 5 | 0 | 0 | 4 | 1 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 4 | 1 | 1 | 4 | 0 | 1 | 4 | | 5 | | | | | | | | | | | | | | | | |
| Sorbitol negative | 3 | 1 | 1 | 1 | 2 | 0 | 4 | 0 | 1 | 3 | 0 | 2 | 2 | 0 | 1 | 2 | 1 | 0 | 4 | 1 | 2 | 1 | | | 4 | | | | | | | | | | | | | | | |
| Dextrin positive | 19 | 0 | 1 | 14 | 4 | 2 | 17 | 0 | 2 | 15 | 2 | 3 | 15 | 1 | 4 | 12 | 3 | 1 | 18 | 3 | 7 | 9 | 15 | 1 | 3 | 19 | | | | | | | | | | | | | | |
| Dextrin late positive | 10 | 2 | 0 | 8 | 4 | 2 | 10 | 0 | 0 | 10 | 2 | 1 | 11 | 0 | 1 | 8 | 3 | 3 | 9 | 0 | 4 | 8 | 7 | 4 | 1 | | 12 | | | | | | | | | | | | | |
| Xylose positive | 17 | 1 | 1 | 13 | 4 | 1 | 17 | 0 | 2 | 15 | 1 | 2 | 15 | 1 | 3 | 12 | 3 | 1 | 17 | 2 | 10 | 6 | 13 | 1 | 4 | 15 | 3 | 18 | | | | | | | | | | | | |
| Xylose late positive | 12 | 1 | 0 | 9 | 4 | 3 | 10 | 0 | 0 | 10 | 3 | 2 | 11 | 0 | 2 | 8 | 3 | 3 | 10 | 1 | 1 | 11 | 9 | 4 | 0 | 4 | 9 | | 13 | | | | | | | | | | | |
| $H_2S$ positive | 20 | 2 | 0 | 17 | 5 | 2 | 20 | 0 | 0 | 19 | 3 | 2 | 20 | 0 | 3 | 16 | 3 | 3 | 19 | 1 | 8 | 13 | 16 | 5 | 1 | 10 | 12 | 11 | 11 | 22 | | | | | | | | | | |
| $H_2S$ late positive | 9 | 0 | 1 | 5 | 3 | 2 | 7 | 0 | 2 | 6 | 1 | 2 | 6 | 1 | 2 | 4 | 3 | 1 | 8 | 2 | 3 | 4 | 6 | 0 | 3 | 9 | 0 | 7 | 2 | | 9 | | | | | | | | | |
| Catalase positive | 26 | 2 | 1 | 21 | 6 | 3 | 25 | 0 | 2 | 22 | 4 | 3 | 24 | 1 | 4 | 20 | 4 | 3 | 25 | 3 | 11 | 14 | 20 | 5 | 3 | 17 | 11 | 16 | 12 | 21 | 7 | 28 | | | | | | | | |
| Catalase negative | 3 | 0 | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 0 | 2 | 1 | 2 | 0 | 0 | 3 | 2 | 0 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | | 3 | | | | | | | |
| V.P test weak positive | 28 | 2 | 1 | 21 | 8 | 3 | 27 | 0 | 2 | 24 | 4 | 4 | 25 | 1 | 5 | 20 | 5 | 3 | 27 | 3 | 11 | 16 | 21 | 5 | 4 | 18 | 12 | 17 | 13 | 22 | 8 | 28 | 2 | 30 | | | | | | |
| V.P test negative | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | | 1 | | | | | |
| Starch from dextrose: positive | 4 | 1 | 0 | 5 | 0 | 1 | 4 | 0 | 0 | 4 | 1 | 1 | 4 | 0 | 1 | 3 | 1 | 1 | 4 | 0 | 3 | 2 | 5 | 0 | 0 | 3 | 2 | 4 | 1 | 4 | 1 | 4 | 1 | 4 | 1 | 5 | | | | |
| Starch from dextrose: weak positive | 6 | 0 | 1 | 3 | 2 | 1 | 5 | 0 | 2 | 4 | 0 | 3 | 3 | 0 | 1 | 4 | 1 | 2 | 4 | 2 | 1 | 3 | 3 | 1 | 2 | 4 | 2 | 4 | 2 | 3 | 3 | 5 | 1 | 6 | 0 | | 6 | | | |
| Starch from dextrose: negative | 19 | 1 | 0 | 14 | 6 | 2 | 18 | 0 | 0 | 17 | 3 | 0 | 19 | 1 | 3 | 13 | 4 | 1 | 19 | 1 | 7 | 12 | 14 | 4 | 2 | 12 | 8 | 10 | 10 | 15 | 5 | 19 | 1 | 20 | 0 | | | 20 | | |
| Starch from maltose: positive | 12 | 0 | 0 | 9 | 3 | 3 | 9 | 0 | 0 | 10 | 2 | 2 | 10 | 0 | 3 | 7 | 2 | 2 | 10 | 2 | 4 | 6 | 8 | 3 | 1 | 7 | 5 | 7 | 5 | 8 | 4 | 10 | 2 | 11 | 1 | 3 | 3 | 6 | 12 | |
| Starch from maltose: weak positive | 9 | 1 | 1 | 6 | 3 | 1 | 9 | 0 | 2 | 7 | 1 | 2 | 8 | 0 | 0 | 8 | 2 | 1 | 9 | 1 | 4 | 5 | 9 | 0 | 1 | 6 | 4 | 6 | 4 | 8 | 2 | 9 | 1 | 10 | 0 | 2 | 3 | 5 | | 10 |
| Starch from maltose: negative | 8 | 1 | 0 | 7 | 2 | 0 | 9 | 0 | 0 | 8 | 1 | 0 | 8 | 1 | 2 | 5 | 2 | 1 | 8 | 0 | 3 | 6 | 5 | 2 | 2 | 6 | 3 | 5 | 4 | 6 | 3 | 9 | 0 | 9 | 0 | 0 | 0 | 9 | | 9 |

TABLE 22. FERMENTATION AND BIOCHEMICAL REACTIONS OF 39 STRAINS OF ACTINOBACILLI ISOLATED FROM THE TONGUES OF NORMAL CATTLE

| | Lactose late positive | Lactose negative | Raffinose late positive | Raffinose negative | Arabinose positive | Arabinose late positive | Arabinose negative | Glycerol positive | Glycerol late positive | Glycerol negative | Galactose positive | Galactose late positive | Galactose negative | Mannose positive | Mannose late positive | Mannose negative | Inositol late positive | Inositol negative | Rhamnose positive | Rhamnose late positive | Rhamnose negative | Sorbitol positive | Sorbitol late positive | Sorbitol negative | Dextrin positive | Dextrin late positive | Xylose positive | Xylose late positive | $H_2S$ positive | $H_2S$ late positive | Catalase positive | Catalase negative | V.P test positive | V.P test weak positive | V.P test negative | Starch from dextrose: positive | Starch from dextrose: weak positive | Starch from dextrose: negative | Starch from maltose: positive | Starch from maltose: weak positive | Starch from maltose: negative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactose late positive | 35 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lactose negative | | 4 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose late positive | 29 | 0 | 29 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose negative | 6 | 4 | | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose positive | 23 | 3 | 19 | 7 | 26 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose late positive | 10 | 0 | 8 | 2 | | 10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose negative | 2 | 1 | 2 | 1 | | | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol positive | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol late positive | 31 | 4 | 25 | 10 | 23 | 9 | 3 | | 35 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol negative | 3 | 0 | 3 | 0 | 2 | 1 | 0 | | | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose positive | 11 | 3 | 8 | 6 | 11 | 2 | 1 | 1 | 13 | 0 | 14 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose late positive | 22 | 1 | 19 | 4 | 13 | 8 | 2 | 0 | 21 | 2 | | 23 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose negative | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | | | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose positive | 8 | 3 | 7 | 4 | 9 | 1 | 1 | 1 | 9 | 1 | 7 | 4 | 0 | 11 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose late positive | 21 | 1 | 17 | 5 | 13 | 7 | 2 | 0 | 22 | 0 | 6 | 15 | 1 | | 22 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose negative | 6 | 0 | 5 | 1 | 4 | 2 | 0 | 0 | 4 | 2 | 1 | 4 | 1 | | | 6 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol late positive | 13 | 3 | 12 | 4 | 9 | 4 | 3 | 0 | 14 | 2 | 5 | 10 | 1 | 4 | 8 | 4 | 16 | | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol negative | 22 | 1 | 17 | 6 | 17 | 6 | 0 | 1 | 21 | 1 | 9 | 13 | 1 | 7 | 14 | 2 | | 23 | | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose positive | 3 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 1 | 2 | 3 | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose late positive | 7 | 0 | 7 | 0 | 7 | 0 | 0 | 0 | 5 | 2 | 4 | 2 | 1 | 3 | 1 | 3 | 3 | 4 | | 7 | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose negative | 25 | 4 | 21 | 8 | 16 | 10 | 3 | 1 | 27 | 1 | 9 | 19 | 1 | 8 | 18 | 3 | 12 | 17 | | | 29 | | | | | | | | | | | | | | | | | | | | |
| Sorbitol positive | 33 | 4 | 29 | 8 | 24 | 10 | 3 | 1 | 33 | 3 | 12 | 23 | 2 | 11 | 20 | 6 | 16 | 21 | 2 | 7 | 28 | 37 | | | | | | | | | | | | | | | | | | | |
| Sorbitol late positive | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 1 | 0 | 1 | | 2 | | | | | | | | | | | | | | | | | | |
| Sorbitol negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 10 | | | | | | | | | | | | | | | | | |
| Dextrin positive | 20 | 1 | 17 | 4 | 10 | 10 | 1 | 0 | 20 | 1 | 8 | 13 | 0 | 6 | 12 | 3 | 8 | 13 | 0 | 3 | 18 | 21 | 0 | 0 | 21 | | | | | | | | | | | | | | | | |
| Dextrin late positive | 15 | 3 | 12 | 6 | 16 | 0 | 2 | 1 | 15 | 2 | 6 | 10 | 2 | 5 | 10 | 3 | 8 | 10 | 3 | 4 | 11 | 16 | 2 | 0 | | 18 | | | | | | | | | | | | | | | |
| Xylose positive | 31 | 4 | 25 | 10 | 25 | 7 | 3 | 1 | 31 | 3 | 14 | 19 | 2 | 11 | 19 | 5 | 13 | 22 | 3 | 7 | 25 | 33 | 2 | 0 | 18 | 17 | 35 | | | | | | | | | | | | | | |
| Xylose late positive | 4 | 0 | 4 | 0 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 3 | 1 | 3 | 1 | 0 | 0 | 4 | 4 | 0 | 0 | 3 | 1 | | 4 | | | | | | | | | | | | | |
| $H_2S$ positive | 7 | 1 | 6 | 2 | 6 | 2 | 0 | 0 | 7 | 1 | 1 | 6 | 1 | 1 | 4 | 3 | 6 | 2 | 2 | 2 | 4 | 8 | 0 | 0 | 1 | 7 | 7 | 1 | 8 | | | | | | | | | | | | |
| $H_2S$ late positive | 28 | 3 | 23 | 8 | 20 | 8 | 3 | 1 | 28 | 2 | 13 | 17 | 1 | 10 | 18 | 3 | 10 | 21 | 1 | 5 | 25 | 29 | 2 | 0 | 20 | 11 | 28 | 3 | | 31 | | | | | | | | | | | |
| Catalase positive | 32 | 4 | 28 | 8 | 26 | 8 | 2 | 1 | 32 | 3 | 13 | 21 | 2 | 11 | 20 | 5 | 15 | 21 | 3 | 7 | 26 | 34 | 2 | 0 | 19 | 17 | 32 | 4 | 8 | 28 | 36 | | | | | | | | | | |
| Catalase negative | 3 | 0 | 1 | 2 | 0 | 2 | 1 | 0 | 3 | 0 | 1 | 2 | 0 | 0 | 2 | 1 | 1 | 2 | 0 | 0 | 3 | 3 | 0 | 0 | 2 | 1 | 3 | 0 | 0 | 3 | | 3 | | | | | | | | | |
| V.P test positive | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | | | | | | | | |
| V.P test weak positive | 27 | 2 | 22 | 7 | 20 | 8 | 1 | 0 | 26 | 3 | 10 | 18 | 1 | 7 | 16 | 6 | 13 | 16 | 3 | 6 | 20 | 28 | 1 | 0 | 16 | 13 | 26 | 3 | 8 | 21 | 26 | 3 | | 29 | | | | | | | |
| V.P test negative | 7 | 2 | 6 | 3 | 6 | 1 | 2 | 1 | 8 | 0 | 4 | 4 | 1 | 4 | 5 | 0 | 3 | 6 | 0 | 1 | 8 | 8 | 1 | 0 | 4 | 5 | 9 | 0 | 0 | 9 | 9 | 0 | | | 9 | | | | | | |
| Starch from dextrose: positive | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 0 | 2 | | | | | |
| Starch from dextrose: weak positive | 10 | 2 | 8 | 4 | 8 | 4 | 0 | 0 | 11 | 1 | 2 | 10 | 0 | 2 | 8 | 2 | 4 | 8 | 2 | 1 | 9 | 12 | 0 | 0 | 6 | 6 | 11 | 1 | 5 | 7 | 11 | 1 | 1 | 10 | 1 | | 12 | | | | |
| Starch from dextrose: negative | 23 | 2 | 19 | 6 | 16 | 6 | 3 | 1 | 23 | 1 | 11 | 13 | 1 | 9 | 13 | 3 | 10 | 15 | 1 | 4 | 20 | 23 | 2 | 0 | 14 | 11 | 22 | 3 | 2 | 23 | 23 | 2 | 0 | 17 | 8 | | | 25 | | | |
| Starch from maltose: positive | 8 | 3 | 6 | 5 | 9 | 1 | 1 | 0 | 10 | 1 | 4 | 7 | 0 | 3 | 7 | 1 | 4 | 7 | 1 | 2 | 8 | 9 | 2 | 0 | 4 | 7 | 11 | 0 | 1 | 10 | 11 | 0 | 0 | 6 | 5 | 0 | 5 | 6 | 11 | | |
| Starch from maltose: weak positive | 10 | 0 | 8 | 2 | 7 | 3 | 0 | 0 | 9 | 1 | 1 | 8 | 1 | 2 | 6 | 2 | 5 | 5 | 1 | 1 | 8 | 10 | 0 | 0 | 5 | 5 | 9 | 1 | 4 | 6 | 10 | 0 | 0 | 9 | 1 | 0 | 4 | 6 | | 10 | |
| Starch from maltose: negative | 17 | 1 | 15 | 3 | 10 | 6 | 2 | 1 | 16 | 1 | 9 | 8 | 1 | 6 | 9 | 3 | 7 | 11 | 1 | 14 | 3 | 18 | 0 | 0 | 12 | 6 | 15 | 3 | 3 | 15 | 15 | 3 | 1 | 14 | 3 | 2 | 3 | 13 | | | 18 |

(ii) Sheep

Eighty-four strains of actinobacilli isolated from the ruminal contents of normal sheep were examined for their fermentative and biochemical activities and all were found to ferment dextrose, laevulose, sucrose, maltose and mannitol promptly, but to give no fermentation of trehalose, inulin, dulcitol and salicin within 14 days. Their ability to grow on MacConkey's medium and to reduce nitrates to nitrites was similar to that of the strains isolated from cattle. The test for hydrogen sulphide production was not performed on the sheep strains, but the tests for indole and ammonia and the methyl red test were negative in every case. The differences observed between strains with the other tests used are shown in Appendix B for the individual organisms and in a summarized form in Table 23. Cross-relationships between the fermentative and biochemical activities of the sheep rumen strains are tabulated in Table 24 (p. 124).

(c) Agglutination reactions

Each of the actinobacillus-like organisms recovered from cattle and sheep was subjected to the

TABLE 23

Biochemical reactions of actinobacilli from the rumens of sheep

| Test | No. of strains | |
|---|---|---|
| | Positive | Negative |
| Fermentation of:- | | |
| Mannose | 37 (22*) | 47 |
| Galactose | 84 (13*) | 0 |
| Arabinose | 84 (14*) | 0 |
| Rhamnose | 9 (7*) | 75 |
| Lactose | 60* | 24 |
| Raffinose | 41 (40*) | 43 |
| Glycerol | 60* | 24 |
| Inositol | 32* | 52 |
| Dextrin | 54 (1*) | 30 |
| Xylose | 79 (7*) | 5 |
| Catalase production | 73 | 11 |
| Vöges-Proskauer test | 25 (7±) | 59 |
| Methylene blue reduction | 81 (7±) | 3 |
| Urease test | 13 (4±) | 71 |
| Starch synthesis from:- | | |
| Dextrose | 59 (18±) | 25 |
| Maltose | 34 (5±) | 50 |

* Late fermentation (negative at 24 hr; positive within 14 days).

± Weakly positive.

TABLE 24. FERMENTATION AND BIOCHEMICAL REACTIONS OF 84 STRAINS OF ACTINOBACILLI ISOLATED FROM THE RUMINAL CONTENTS OF NORMAL SHEEP

| | Lactose late positive | Lactose negative | Raffinose positive | Raffinose late positive | Raffinose negative | Arabinose positive | Arabinose late positive | Arabinose negative | Glycerol positive | Glycerol late positive | Glycerol negative | Galactose positive | Galactose late positive | Galactose negative | Mannose positive | Mannose late positive | Mannose negative | Inositol late positive | Inositol negative | Rhamnose positive | Rhamnose late positive | Rhamnose negative | Sorbitol positive | Sorbitol late positive | Sorbitol negative | Dextrin positive | Dextrin late positive | Dextrin negative | Xylose positive | Xylose late positive | Xylose negative | Catalase positive | Catalase negative | V.P. test positive | V.P. test weak positive | V.P. test negative | Starch from dextrose positive | Starch from dextrose weak positive | Starch from dextrose negative | Starch from maltose positive | Starch from maltose weak positive | Starch from maltose negative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactose late positive | 60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lactose negative | | 24 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose positive | 1 | 0 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose late positive | 34 | 6 | | 40 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Raffinose negative | 25 | 18 | | | 43 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose positive | 52 | 18 | 1 | 33 | 36 | 70 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose late positive | 8 | 6 | 0 | 7 | 7 | | 14 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arabinose negative | 0 | 0 | 0 | 0 | 0 | | | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol positive | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol late positive | 47 | 13 | 1 | 33 | 26 | 55 | 5 | 0 | | 60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glycerol negative | 13 | 11 | 0 | 7 | 17 | 15 | 9 | 0 | | | 24 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose positive | 51 | 20 | 1 | 34 | 36 | 58 | 13 | 0 | 0 | 51 | 20 | 71 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose late positive | 9 | 4 | 0 | 6 | 7 | 12 | 1 | 0 | 0 | 9 | 4 | | 13 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Galactose negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose positive | 14 | 1 | 0 | 3 | 12 | 13 | 2 | 0 | 0 | 12 | 3 | 14 | 1 | 0 | 15 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose late positive | 15 | 7 | 1 | 14 | 7 | 18 | 4 | 0 | 0 | 20 | 2 | 19 | 3 | 0 | | 22 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mannose negative | 31 | 16 | 0 | 23 | 24 | 39 | 8 | 0 | 0 | 28 | 19 | 38 | 9 | 0 | | | 47 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol late positive | 21 | 11 | 0 | 21 | 11 | 30 | 2 | 0 | 0 | 28 | 4 | 28 | 4 | 0 | 2 | 13 | 17 | 32 | | | | | | | | | | | | | | | | | | | | | | | | | |
| Inositol negative | 39 | 13 | 1 | 19 | 32 | 40 | 12 | 0 | 0 | 32 | 20 | 43 | 9 | 0 | 13 | 9 | 30 | | 52 | | | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose positive | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose late positive | 7 | 0 | 0 | 3 | 4 | 6 | 1 | 0 | 0 | 7 | 0 | 7 | 0 | 0 | 3 | 4 | 0 | 1 | 6 | | 7 | | | | | | | | | | | | | | | | | | | | | | |
| Rhamnose negative | 51 | 24 | 1 | 36 | 38 | 62 | 13 | 0 | 0 | 51 | 24 | 64 | 11 | 0 | 12 | 17 | 46 | 30 | 45 | | | 75 | | | | | | | | | | | | | | | | | | | | | |
| Sorbitol positive | 58 | 22 | 1 | 38 | 41 | 67 | 13 | 0 | 0 | 56 | 24 | 67 | 13 | 0 | 14 | 20 | 46 | 29 | 51 | 2 | 7 | 71 | 80 | | | | | | | | | | | | | | | | | | | |
| Sorbitol late positive | 2 | 2 | 0 | 2 | 2 | 3 | 1 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 1 | 2 | 1 | 3 | 1 | 0 | 0 | 4 | | 4 | | | | | | | | | | | | | | | | | | |
| Sorbitol negative | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | 0 | | | | | | | | | | | | | | | | | |
| Dextrin positive | 33 | 20 | 0 | 26 | 27 | 44 | 9 | 0 | 0 | 38 | 15 | 46 | 7 | 0 | 12 | 15 | 26 | 22 | 31 | 1 | 5 | 47 | 49 | 4 | 0 | 53 | | | | | | | | | | | | | | | |
| Dextrin late positive | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | | 1 | | | | | | | | | | | | | | |
| Dextrin negative | 26 | 4 | 1 | 13 | 16 | 26 | 4 | 0 | 0 | 22 | 8 | 25 | 5 | 0 | 2 | 7 | 21 | 10 | 20 | 1 | 2 | 27 | 30 | 0 | 0 | | | 30 | | | | | | | | | | | | | |
| Xylose positive | 50 | 22 | 0 | 34 | 38 | 59 | 13 | 0 | 0 | 50 | 22 | 64 | 8 | 0 | 13 | 17 | 42 | 30 | 42 | 1 | 3 | 68 | 68 | 4 | 0 | 46 | 1 | 25 | 72 | | | | | | | | | | | | |
| Xylose late positive | 6 | 1 | 0 | 5 | 2 | 6 | 1 | 0 | 0 | 6 | 1 | 4 | 3 | 0 | 0 | 3 | 4 | 2 | 5 | 1 | 2 | 4 | 7 | 0 | 0 | 3 | 0 | 4 | | 7 | | | | | | | | | | | |
| Xylose negative | 4 | 1 | 1 | 1 | 3 | 5 | 0 | 0 | 0 | 4 | 1 | 3 | 2 | 0 | 2 | 2 | 1 | 0 | 5 | 0 | 2 | 3 | 5 | 0 | 0 | 4 | 0 | 1 | | | 5 | | | | | | | | | |
| Catalase positive | 50 | 23 | 1 | 39 | 33 | 60 | 13 | 0 | 0 | 51 | 22 | 60 | 13 | 0 | 4 | 22 | 47 | 31 | 42 | 2 | 4 | 67 | 70 | 3 | 0 | 42 | 1 | 30 | 63 | 7 | 3 | 73 | | | | | | | | | |
| Catalase negative | 10 | 1 | 0 | 1 | 10 | 10 | 1 | 0 | 0 | 9 | 2 | 11 | 0 | 0 | 11 | 0 | 0 | 1 | 10 | 0 | 3 | 8 | 10 | 1 | 0 | 11 | 0 | 0 | 9 | 0 | 2 | | 11 | | | | | | | |
| V.P. test positive | 16 | 2 | 1 | 5 | 12 | 15 | 3 | 0 | 0 | 10 | 8 | 12 | 6 | 0 | 4 | 4 | 10 | 2 | 16 | 1 | 1 | 16 | 17 | 1 | 0 | 7 | 0 | 11 | 15 | 1 | 2 | 15 | 3 | 18 | | | | | | |
| V.P. test weak positive | 7 | 0 | 0 | 4 | 3 | 6 | 1 | 0 | 0 | 6 | 1 | 7 | 0 | 0 | 2 | 2 | 3 | 1 | 6 | 0 | 1 | 6 | 7 | 0 | 0 | 5 | 0 | 2 | 7 | 0 | 0 | 5 | 2 | | 7 | | | | | |
| V.P. test negative | 37 | 22 | 0 | 31 | 28 | 49 | 10 | 0 | 0 | 44 | 15 | 52 | 7 | 0 | 9 | 16 | 34 | 29 | 30 | 1 | 5 | 53 | 56 | 3 | 0 | 41 | 1 | 17 | 50 | 6 | 3 | 53 | 6 | | | 59 | | | | |
| Starch from dextrose positive | 29 | 12 | 0 | 15 | 26 | 32 | 9 | 0 | 0 | 27 | 14 | 37 | 4 | 0 | 11 | 5 | 25 | 10 | 31 | 0 | 3 | 38 | 39 | 2 | 0 | 20 | 1 | 20 | 37 | 3 | 1 | 33 | 8 | 10 | 3 | 28 | 41 | | | |
| Starch from dextrose weak positive | 14 | 4 | 1 | 9 | 8 | 15 | 3 | 0 | 0 | 9 | 9 | 14 | 4 | 0 | 3 | 6 | 9 | 5 | 13 | 1 | 3 | 14 | 17 | 1 | 0 | 11 | 0 | 7 | 12 | 3 | 3 | 15 | 3 | 6 | 1 | 11 | | 18 | | |
| Starch from dextrose negative | 17 | 8 | 0 | 16 | 9 | 23 | 2 | 0 | 0 | 24 | 1 | 20 | 5 | 0 | 1 | 11 | 13 | 17 | 8 | 1 | 1 | 23 | 24 | 1 | 0 | 22 | 0 | 3 | 23 | 1 | 1 | 25 | 0 | 2 | 3 | 20 | | | 25 | |
| Starch from maltose positive | 19 | 10 | 0 | 11 | 18 | 22 | 7 | 0 | 0 | 17 | 12 | 23 | 6 | 0 | 11 | 3 | 15 | 6 | 23 | 1 | 2 | 26 | 28 | 1 | 0 | 26 | 1 | 2 | 25 | 2 | 2 | 19 | 10 | 7 | 3 | 19 | 19 | 7 | 3 | 29 |
| Starch from maltose weak positive | 3 | 2 | 0 | 3 | 2 | 4 | 1 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 1 | 3 | 1 | 3 | 2 | 0 | 2 | 3 | 3 | 2 | 0 | 5 | 0 | 0 | 3 | 1 | 1 | 4 | 1 | 0 | 0 | 5 | 2 | 2 | 1 | |
| Starch from maltose negative | 38 | 12 | 1 | 26 | 23 | 44 | 6 | 0 | 0 | 38 | 12 | 43 | 7 | 0 | 3 | 16 | 31 | 23 | 27 | 1 | 3 | 46 | 49 | 1 | 0 | 22 | 0 | 28 | 44 | 4 | 2 | 50 | 0 | 11 | 4 | 35 | 20 | 9 | 21 | |

slide agglutination test with 27 antisera (antiserum A8 was omitted) prepared against strains of A. lignieresi isolated from pathological material (Part II, p. 73) and with the two antisera against the normal bovine rumen actinobacilli, strains 43.4 and 97B3. The results of these tests with individual organisms are given in Appendix C (pp. 264-285). The number of strains which were agglutinated by each of the antisera are shown in Table 25. The incidence of strains of actinobacillus-like organisms which were not agglutinated by any of the A. lignieresi antisera is set out in Table 26 (p. 127) and of those which were agglutinated by the two antisera against the rumen strains in Table 27 (p. 127).

The majority of the strains which were agglutinated by the A. lignieresi antisera did not fall clearly into the antigenic types into which the strains from pathological material could be divided. A small number of strains, however, did coincide with these types, e.g. the lingual strain M98.1 and the sheep rumen strains 4/4/5 and 10/8/5 are of antigenic type 4, the bovine rumen strain 15.1 of antigenic type 1 and the two sheep rumen strains 8/29/5 and 2/19/6 of antigenic type 3.

TABLE 25

Agglutination of commensal actinobacilli

| Source | Bovine Rumen | Bovine Tongue | Ovine Rumen |
|---|---|---|---|
| Antiserum against strain no. | No.of strains agglutinated | No.of strains agglutinated | No.of strains agglutinated |
| A1 | 1 | 0 | 0 |
| A2 | 1 | 0 | 2 |
| A4 | 2 | 0 | 1 |
| A19 | 1 | 0 | 0 |
| A22 | 2 | 1 | 2 |
| A46 | 1 | 0 | 1 |
| A47 | 4 | 4 | 9 |
| A55 | 1 | 0 | 0 |
| A56 | 1 | 0 | 0 |
| A60 | 4 | 0 | 1 |
| A100 | 1 | 0 | 2 |
| A133 | 0 | 0 | 2 |
| A3 | 1 | 1 | 7 |
| A5 | 1 | 0 | 7 |
| A6 | 1 | 0 | 5 |
| A14 | 0 | 1 | 5 |
| A7 | 1 | 2 | 11 |
| A33 | 0 | 2 | 9 |
| A53 | 8 | 16 | 6 |
| A13 | 18 | 22 | 38 |
| A43 | 1 | 0 | 0 |
| A59 | 0 | 0 | 0 |
| A75 | 0 | 0 | 0 |
| A15 | 0 | 0 | 0 |
| A17 | 0 | 0 | 2 |
| A49 | 4 | 5 | 16 |
| A20 | 1 | 0 | 3 |
| 43.4 | 11 | 19 | 7 |
| 97B3 | 11 | 16 | 0 |

TABLE 26

Agglutination of commensal actinobacilli in slide tests with antisera against A. lignieresi strains

| Source | No. of strains agglutinated by one or more sera | No. of strains not agglutinated by any serum | No. of strains autoagglutinating |
|---|---|---|---|
| Bovine rumen | 22 | 8 | 1 |
| Bovine tongue | 25 | 12 | 2 |
| Ovine rumen | 51 | 33 | 0 |

TABLE 27

Agglutination of commensal actinobacilli in slide tests with antisera against 2 rumen strains

| Source | No. of strains agglutinated by antiserum | | |
|---|---|---|---|
| | 43.4 | 97B3 | Both |
| Bovine rumen | 0 | 0 | 11 |
| Bovine tongue | 3 | 0 | 16 |
| Ovine rumen | 7 | 0 | 0 |

The antisera which agglutinated the highest proportion of strains from all three sources were A13, A53 and A49. It is interesting to note that many of the strains which agglutinated with antisera A13 and A53 were also agglutinated by the antiserum against the rumen actinobacillus strain 43.4

The heated slide antigens of 56 of the strains of A. lignieresi isolated from pathological material were also tested with the two antisera prepared against the rumen actinobacilli. The distribution of these strains among the antigenic types as well as the results with the two new antisera are given in Table 28. The notable feature of these results is that all except one of the type 4 strains of A. lignieresi were agglutinated by serum 43.4 and, moreover, two-thirds of the strains which were agglutinated by this serum were of this antigenic type. Organisms of antigenic type 4 are agglutinated by antisera A53 and A13.

Four strains of A. lignieresi which had been found to agglutinate with antiserum 43.4 were used to absorb this antiserum. Each absorbed serum was then tested by the slide test against heated slide antigens of each of the absorbing strains as well as strain

TABLE 28

Agglutination of 56 strains of A. lignieresi by 2 antisera against rumen actinobacilli

| Antigenic type | No.of strains of antigenic type | No.of strains agglutinated by antiserum 43.4 | No.of strains agglutinated by antiserum 97B3 |
|---|---|---|---|
| 1 | 29 | 0 | 0 |
| 1a | 4 | 0 | 0 |
| 2 | 2 | 0 | 0 |
| 3 | 2 | 0 | 0 |
| 4 | 7 | 6 | 0 |
| 4a | 1 | 0 | 0 |
| 5 | 4 | 0 | 0 |
| 6 | 2 | 1 | 0 |
| Untyped (A4) | 4 | 2 | 0 |
| Untyped | 1 | 0 | 0 |

43.4. Strain A13 was also used to absorb antiserum 43.4 since, although it was not agglutinated by this antiserum, antiserum A13 agglutinated strain 43.4. Antisera A53, A7 and A13 were absorbed with strain 43.4 and, in addition, antiserum A53 was absorbed with strain N.C.T.C. 4189. All these absorbed sera were then tested against all the strains used for absorption. The results of these tests are set out in Table 29. Although the antibodies against the homologous organism of antiserum 43.4 were not removed

TABLE 29

Absorption of 4 antisera with A. lignieresi strains and 1 rumen actinobacillus strain

| Antiserum against strain | Absorbed with strain | Tested against heated slide antigen of strain:- | | | | | |
|---|---|---|---|---|---|---|---|
| | | 43.4 | A7 | A13 | A53 | NCTC 4189 | A15 |
| 43.4 | Unabsorbed | + | + | - | + | + | + |
| | A7 | + | - | ... | - | + | + |
| | A13 | + | + | ... | + | + | + |
| | A53 | + | - | ... | - | + | + |
| | NCTC 4189 | + | ... | ... | - | - | + |
| | A15 | - | ... | ... | - | - | - |
| A53 | Unabsorbed | + | + | - | + | + | + |
| | 43.4 | - | + | ... | + | + | - |
| | NCTC 4189 | - | ... | ... | + | - | - |
| A7 | Unabsorbed | - | + | - | + | + | - |
| | 43.4 | ... | + | ... | + | - | ... |
| A13 | Unabsorbed | + | + | + | + | + | - |
| | 43.4 | - | + | + | + | + | ... |

+ = agglutination

- = no agglutination

by absorption with strains A7 and A53, the antibodies against both of these strains were removed. Strain A15, however, absorbed the serum completely and left it devoid of agglutinating activity against all the antigens tested. A degree of relationship between 43.4 and N.C.T.C. 4189 is apparent from these results, since 43.4 removed antibodies against the N.C.T.C. strain from antiserum A7, and N.C.T.C. 4189 removed antibodies against 43.4 from antiserum A53.

## 4. DISCUSSION

The comparative ease with which actinobacilli were recovered from both the tongue and the ruminal contents of cattle suggests that they may be multiplying in these sites. It is likely that the percentage of animals from which these organisms were recovered is too low, since there is considerable immunological evidence that the majority of adult cattle have had some antigenic experience of A. lignieresi (see Part IV, p. 146 ). It is unlikely, moreover, that the animals from which the positive cultures were obtained represent undetected cases of actinobacillosis, since the incidence of this disease in cattle slaughtered during the past 10 years at Edinburgh Abattoir, from which the material was drawn, has varied between 0.58 and 1.01 per cent. (Edinburgh Medical Officer of Health, 1955, 1956, 1957, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965).

The failure of Till and Palmer (1960) to isolate A. lignieresi from the tongues of 180 cattle is surprising in view of the percentage recovery obtained in the present work. These workers employed an antibiotic digest agar containing oleandomycin (20 µg/ml.) and neomycin (1.5 µg/ml.). It is possible

that this medium may have been too inhibitory to the actinobacilli, since the concentration of oleandomycin is close to the minimal inhibitory concentration of this agent for A. lignieresi determined in the present work. Moreover, Till and Palmer relied upon agglutination of their isolates by 2 antisera as a determinative factor in their examination, and this would have excluded most of the strains recovered in this investigation.

There are few records of the sensitivity of A. lignieresi to antibiotics. Williams-Smith (1951) found, with in-vitro tests, that cultures of the organism were only slightly sensitive to penicillin, a fact which was confirmed by Ristic et al. (1956). These latter workers examined the activity of other antibiotics and found all strains to be sensitive to chloromycetin, aureomycin and terramycin. The results of Till and Palmer (1960) using a wider range of antibiotics were confirmed in the present work.

A feature of the biochemical results was the slightly greater fermentative activity of the organisms recovered from normal cattle and sheep compared with strains isolated from lesions. Although only 22 per cent. of these latter strains fermented raffinose, 74

and 48 per cent. of strains isolated from normal cattle and sheep, respectively, fermented this substrate. With rhamnose also there was a considerably greater proportion of the rumen and tongue strains which brought about fermentation than was the case with strains from lesions. In contrast with this, fermentation of the two hexoses, mannose and galactose, was less vigorous with the actinobacillus-like organisms than with A. lignieresi. Late fermentation of galactose has been reported (Till and Palmer, 1960), however, and variable results with mannose are not unknown (Ristic et al., 1956; Pathak and Ristic, 1962). Although none of the strains isolated from lesions attacked inositol, between 13 and 41 per cent. of the rumen and lingual organisms did so. The strains of A. lignieresi reported by Till and Palmer (1960) gave late fermentation of inositol.

The production of catalase by 87 to 92 per cent. of the actinobacillus-like organisms isolated from cattle and sheep differs markedly from the 5.5 per cent. of A. lignieresi which produced this enzyme. This, however, is in accordance with the findings of Ristic et al. (1956) and Till and Palmer (1960) for their strains of A. lignieresi.

A surprising feature of the results with the tests for starch-production was that strains from the bovine rumen and tongue were less active in this respect than the strains from lesions. This was also observed with sheep strains, although the difference with starch production from dextrose was less.

If, as has already been suggested, actinobacilli may form part of the iodophilic flora of the rumen and be concerned in microbial digestion in that organ, the slight differences in in-vitro biochemical activities between the "normal" and the pathogenic strains may indicate the more active fermentative role required of the commensal form.

The very limited antigenic examination of the strains isolated from normal ruminal contents and tongues has shown that, although some of the antigenic types recognised in strains of A. lignieresi from pathological material are represented amongst the normal strains, the majority cannot be typed in this way. The cross-agglutination obtained with many of the normal strains is of interest, particularly since the antisera which most frequently brought about these reactions (A13, A49 and A53) are those which caused the greatest degree of cross-agglutination with

A. lignieresi strains (Part II, pp. 79-80). Another interesting feature is the cross reactions which were seen with antiserum 43.4 and the normal strains. Of the strains isolated from cattle 43 per cent. agglutinated with this serum, although the proportion of the sheep strains which did so was very much less (8 per cent.).

A more thorough antigenic analysis of the strains of actinobacilli derived from normal cattle and sheep will be necessary to determine the exact position of these organisms, but the results reported here clearly show that A. lignieresi does occur as a commensal organism and that there exist in the normal ruminal contents of cattle and sheep actinobacillus-like organisms, some of which are antigenically related to A. lignieresi whilst others have not, so far, exhibited any such relationships. It is possible that, as with other groups of organisms, there are many antigenic types of actinobacilli of which only a few are pathogenic. This, however, can be determined only by an extensive survey.

## Part IV: The incidence of antibodies to *Actinobacillus lignieresi* in the sera of cattle and sheep

## 1. INTRODUCTION

Actinobacillosis of the tongue and cheek present no great problems in clinical diagnosis and, since the response to treatment with iodides, sulphonamide drugs and antibiotics is usually rapid (Tutt, 1935; Farquharson, 1937; Dawson, 1945; Barrett, 1949; Smrěck, 1952; Bruere, 1955; Čollák and Orsàg, 1957-8), the clinician does not often fall back on laboratory aids for his diagnosis. However, when other tissues such as the oesophagus, rumen, reticulum, lung or liver are involved, differential diagnosis may be difficult and the use of a serological test as a diagnostic aid would be of value in some cases.

Lignières and Spitz (1902), describing the characters of the organism and the disease stated that "La séro-agglutination est ..... un moyen de diagnostic applicable à l'actinobacillose; ses résultats seront surtout exacts si l'on procède par comparaison, en employant, en même temps que le sérum à essayer, deux sérums témoins: l'un agglutinant, l'autre normal.". They did not give any quantitative estimation of the amounts of antibody to be expected except that the addition of about 0.25 cc. of the serum to "a tube of broth culture" caused agglutination

to appear within 15-30 minutes and to be complete after 2 hours, whereas serum from a normal animal only brought about agglutination after a much longer time (8-10 hours). Pathak and Ristic (1962), however, found that the whole cell agglutination test was unsuitable for the detection of blood serum antibody.

The agglutination test is recommended by Wilson and Miles (1955, p. 1470) who state that the sera of infected animals often agglutinate the organism at a titre of 1 in 50 or over, and by Davies (1955, p. 310) who gives a range of titres for infected animals of 1 in 60 to 1 in 2500. Thompson (1933a) working in America had doubts about the value of the agglutination test as a completely satisfactory diagnostic method for cattle. Hebeler et al. (1961) found that most of the infected cattle in the herd outbreak which they described had serum titres against the infecting organism of 1 in 32 or 1 in 64 and control animals had titres only marginally lower than this.

The agglutination test has also been used in the investigation of the disease in sheep. Magnusson (1929) showed that, whilst the serum from naturally infected sheep would agglutinate suspensions of A. lignieresi at dilutions ranging from 1 in 320 to

1 in 1280, the serum of healthy animals did so only at dilutions of 1 in 20 to 1 in 100. Such distinct differences between normal and infected sheep were not observed by Tunnicliff (1941) who concluded that the organism must be more widespread than had been recognised previously and that this resulted in the sensitisation of the general sheep population. Taylor (1944) compared the serum titres of normal and infected sheep when tested with suspensions of A. lignieresi and showed that infected animals had appreciably higher levels of antibody.

The divergent results of previous workers, the absence of any extensive examination of sera from normal animals and failure in earlier studies to adequately identify the antigenic types of A. lignieresi prompted the investigation described in this section of the work. It was the purpose to test a considerable number of sera from normal cattle and sheep with suspensions of A. lignieresi representing distinct antigenic types, and to compare these results with those obtained in similar tests on the sera of animals known to be affected with actinobacillosis.

## 2. MATERIALS AND METHODS

### (a) Media

The media used in the preparation of antigens were those already described in Part II (p. 70). Attempts were made to isolate A. lignieresi from swabs taken from the tongues of clinical cases of actinobacillosis, using the horse blood agar containing oleandomycin and nystatin described in Part III (p. 114).

### (b) Preparation of antigens

Bacterial suspensions for use in agglutination tests were prepared in the same way as the "heated antigen" and "formolised antigen" in Part II (p. 71).

### (c) Collection of sera

Blood from adult cattle slaughtered at Edinburgh Abattoir was collected either from the axillary vessels or from the chambers of the heart when this organ was opened for inspection. Tissues were taken for cultural examination from any animal showing lesions with the macroscopic appearance of actinobacillosis. Two hundred and eight sera submitted to the Department for examination for contagious bovine abortion were also included in this work. Twenty-one young cattle

in the herd attached to the Veterinary School were bled from the jugular vein at monthly intervals over a period of 20 months. One hundred and seventy-three samples of blood from cattle clinically affected with actinobacillosis were submitted by practising veterinary surgeons.

Blood from 330 normal sheep was collected from the severed neck vessels at the time of slaughter.

(d) Strains of A. lignieresi

At the time this section of the work was carried out the antigenic studies on A. lignieresi, although begun, were not completed, but it had become apparent that several antigenic types of A. lignieresi existed. Five strains of A. lignieresi (A2, A3, A7, A13 and A15) isolated from pathological material were used in the preparation of antigens.

(e) Agglutination tests

Tube agglutination tests were carried out by the same technique used in Part II (p. 75) of this work. The first serum dilution was 1 in 10 in every case, but the range varied with the purpose of the test. Sera from normal animals were examined initially in the range 1 in 10 to 1 in 160, and any sera showing

agglutination at 1 in 160 were further examined in a dilution series from 1 in 10 to 1 in 20,480. In addition, a number of sera with titres less than 1 in 160 were examined in this range of dilutions to test for the presence of a prozone.

Sera from clinical cases of actinobacillosis and from animals showing lesions at meat inspection were examined over the range 1 in 10 to 1 in 20,480.

## 3. RESULTS

### (a) Normal cattle

#### (i) Adult animals

Five hundred and fifty-two slaughterhouse samples from normal cattle were collected and subjected to agglutination tests using heated antigens of the five strains of A. lignieresi. Included in this group were 189 animals from which ruminal contents were also collected for examination, the results of which have been recorded in Part III. The results of the agglutination tests are given in Table 30. Prozones were found in only 4 tests and 3 of these occurred with one serum. The highest titre occurring with a prozone was 1 in 320 with antigen A2. In all cases the prozone was not extensive and, in two cases, the titre was only 1 in 80.

The results obtained with 208 blood samples submitted to the laboratory for examination for contagious bovine abortion are given in Table 31. Prozones were present on only two occasions and both of these were obtained with the same serum, the titre with each antigen being 1 in 160. One serum brought about agglutination at a dilution higher than 1 in 320, the

TABLE 30

Agglutination titres of 552 samples of normal bovine serum taken at slaughter in tests against heated suspensions of five strains of A. lignieresi

| Strain tested as agglutinable suspension | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|
| | | <10 | 10-20 | 40-160 | 320 | >320 |
| A2 | 1 | 279 | 231 | 40 (4) | 2 | 0 |
| A3 | 0 | 14 | 99 | 439 (74) | 0 | 0 |
| A7 | 0 | 71 | 387 | 94 (2) | 0 | 0 |
| A13 | 1 | 191 | 317 | 44 (0) | 0 | 0 |
| A15 | 2 | 310 | 217 | 24 (1) | 1 | 0 |

TABLE 31

Agglutination titres of 208 samples of bovine serum collected for examination for contagious bovine abortion in tests against heated suspensions of five strains of A. lignieresi

| Strain tested as agglutinable suspension | No. of sera showing prozone | No. of sera showing titre* at | | | | |
|---|---|---|---|---|---|---|
| | | <10 | 10-20 | 40-160 | 320 | >320 |
| A2 | 0 | 47 | 110 | 51 (0) | 0 | 0 |
| A3 | 1 | 2 | 20 | 186 (26) | 0 | 0 |
| A7 | 1 | 13 | 91 | 104 (5) | 0 | 0 |
| A13 | 0 | 37 | 81 | 90 (2) | 0 | 0 |
| A15 | 0 | 41 | 81 | 84 (2) | 1 | 1 |

* Titres are reciprocals of highest dilution of serum giving agglutination of the test strain of actinobacillus. Figures in brackets are numbers of sera giving complete agglutination at a dilution of 1 in 160.

titre being 1 in 1280. The animals from which these laboratory samples were derived have been assumed not to be clinical cases of actinobacillosis, but no post-mortem examinations were available to confirm this.

With both slaughterhouse and laboratory samples, the majority of sera gave agglutination at dilutions no higher than 1 in 20, with the exception that antigen A3, which appeared to be especially sensitive, was agglutinated by most of the sera in dilutions of from 1 in 40 to 1 in 160. In no case, however, did agglutination occur at a higher dilution than 1 in 160 with antigen A3. In two cases, each with antigens A2 and A15, agglutination occurred at a dilution of 1 in 320. Very few sera in these two groups were without any activity whatsoever on the antigens employed; only 4 laboratory samples from calves and 8 slaughterhouse samples gave completely negative results.

The use of formolised antigens to examine sera from clinical cases was introduced some time after the investigation was begun and therefore not all sera from normal animals were tested with these antigens. However, a comparison of the two types of antigen,

using only 4 antigenic strains (omitting A15), was made with 92 slaughterhouse samples selected at random. The results of these tests are shown in Table 32. The general result shows a similarity in the reactions with the two types of antigen, the formolised antigens being slightly more sensitive than the heated ones. In no case was there more than one dilution tube difference in the results with the two types of antigen.

TABLE 32

Agglutination titres of 92 sera collected from normal cattle at slaughter in tests against four heated and four formolised suspensions of A. lignieresi

| Agglutinable suspension | | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|
| Preparation | Strain | | <10 | 10-20 | 40-160 | 320 | >320 |
| Heated ..... | A2 | 0 | 35 | 51 | 6 (0) | 0 | 0 |
| | A3 | 1 | 1 | 12 | 79 (1) | 0 | 0 |
| | A7 | 0 | 9 | 69 | 14 (0) | 0 | 0 |
| | A13 | 0 | 23 | 62 | 7 (0) | 0 | 0 |
| Formolised .. | A2 | 0 | 21 | 63 | 8 (0) | 0 | 0 |
| | A3 | 0 | 0 | 15 | 76 (1) | 1 | 0 |
| | A7 | 0 | 6 | 75 | 11 (1) | 0 | 0 |
| | A13 | 0 | 26 | 61 | 5 (0) | 0 | 0 |

* See footnote to Table 31.

(ii) Young animals

The absence of antibodies to A. lignieresi in the 4 laboratory samples from calves prompted a more extensive examination of samples from young stock. Blood samples were drawn at monthly intervals from all calves in the dairy herd attached to the Veterinary School and were examined against heated antigens. The results for the individual animals sampled are shown in Appendix D. Table 33 shows the results for various age groups and includes a score based upon the agglutination results with all five antigens. The system of scoring is as follows:- complete agglutination at a dilution of 1 in 10 scores 2 marks; at 1 in 20, 4 marks; at 1 in 40, 6 marks; at 1 in 80, 8 marks; at 1 in 160, 10 marks; incomplete agglutination at any dilution scores 1 mark less than with complete agglutination. No serum agglutinated any antigen at a dilution higher than 1 in 160 and no prozone was observed.

Antibodies to A. lignieresi were absent from the sera of very young calves (1-2 weeks) but with increasing age in the animals a gradual increase of such antibodies was noticed, the titres reaching their maxima (ca. 20 marks) at about 1 year of age.

TABLE 33

Agglutination titres of sera from 21 young cattle at various ages in tests against heated suspensions of five strains of A. lignieresi

| Age of animals (wk) | No. of sera showing stated titre in test with strain | | | | | | | | | | | | | | | No. of animals examined | Average score for animals examined (marks)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A2 | | | A3 | | | A7 | | | A13 | | | A15 | | | | |
| | <10 | 10-20 | 40-160 | <10 | 10-20 | 40-160 | <10 | 10-20 | 40-160 | <10 | 10-20 | 40-160 | <10 | 10-20 | 40-160 | | |
| 0 - 4 | 10 | 2 | 0 | 12 | 0 | 0 | 12 | 0 | 0 | 10 | 1 | 1 | 11 | 1 | 0 | 12 | 1.0 |
| 5 - 8 | 9 | 10 | 0 | 17 | 2 | 0 | 17 | 2 | 0 | 17 | 2 | 0 | 14 | 5 | 0 | 19 | 1.6 |
| 9 - 12 | 8 | 11 | 0 | 11 | 6 | 2 | 19 | 0 | 0 | 14 | 5 | 0 | 14 | 5 | 0 | 19 | 2.9 |
| 13 - 16 | 11 | 6 | 0 | 1 | 13 | 3 | 11 | 6 | 0 | 11 | 6 | 0 | 13 | 4 | 0 | 17 | 5.2 |
| 17 - 20 | 9 | 8 | 1 | 0 | 10 | 8 | 9 | 8 | 1 | 13 | 5 | 0 | 11 | 7 | 0 | 18 | 7.9 |
| 21 - 24 | 4 | 9 | 0 | 1 | 6 | 6 | 4 | 9 | 0 | 5 | 7 | 1 | 9 | 4 | 0 | 13 | 10.4 |
| 25 - 28 | 7 | 13 | 0 | 1 | 8 | 11 | 7 | 13 | 0 | 8 | 10 | 2 | 9 | 9 | 2 | 20 | 10.2 |
| 29 - 32 | 5 | 11 | 0 | 0 | 4 | 12 | 1 | 12 | 3 | 5 | 9 | 2 | 10 | 5 | 1 | 16 | 14.3 |
| 33 - 36 | 6 | 9 | 1 | 0 | 4 | 12 | 0 | 14 | 2 | 3 | 11 | 2 | 5 | 11 | 0 | 16 | 14.5 |
| 37 - 40 | 4 | 9 | 0 | 0 | 3 | 10 | 1 | 11 | 1 | 4 | 8 | 1 | 7 | 6 | 0 | 13 | 12.3 |
| 41 - 44 | 5 | 9 | 1 | 0 | 2 | 13 | 2 | 9 | 4 | 1 | 10 | 4 | 6 | 7 | 2 | 15 | 16.7 |
| 45 - 48 | 4 | 7 | 1 | 0 | 4 | 8 | 0 | 11 | 1 | 2 | 9 | 1 | 5 | 7 | 0 | 12 | 13.8 |
| 49 - 52 | 1 | 7 | 1 | 0 | 2 | 7 | 0 | 8 | 1 | 0 | 9 | 0 | 2 | 7 | 0 | 9 | 16.8 |
| 53 - 56 | 2 | 10 | 0 | 0 | 1 | 11 | 0 | 9 | 3 | 1 | 9 | 2 | 2 | 8 | 2 | 12 | 19.8 |
| 57 - 60 | 1 | 12 | 0 | 0 | 0 | 13 | 0 | 9 | 4 | 0 | 9 | 4 | 2 | 6 | 5 | 13 | 22.0 |
| 61 - 64 | 2 | 8 | 1 | 0 | 0 | 11 | 1 | 9 | 1 | 2 | 7 | 2 | 0 | 8 | 3 | 11 | 19.5 |
| 65 - 68 | 1 | 10 | 1 | 0 | 1 | 11 | 0 | 8 | 4 | 2 | 8 | 2 | 2 | 9 | 1 | 12 | 20.1 |
| 69 - 72 | 2 | 7 | 0 | 0 | 2 | 7 | 0 | 6 | 3 | 2 | 5 | 2 | 0 | 6 | 3 | 9 | 19.2 |
| 73 - 76 | 1 | 3 | 1 | 0 | 0 | 5 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 3 | 2 | 5 | 21.2 |
| 77 - 80 | 3 | 6 | 2 | 0 | 1 | 10 | 0 | 6 | 5 | 4 | 4 | 3 | 0 | 5 | 6 | 11 | 22.2 |
| 81 - 84 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 3 | 0 | 0 | 1 | 2 | 3 | 24.0 |
| 85 - 88 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 22.5 |
| 89 - 92 | 2 | 2 | 1 | 0 | 1 | 4 | 0 | 2 | 3 | 4 | 0 | 1 | 0 | 4 | 1 | 5 | 19.6 |
| 93 - 96 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 10.0 |
| 97 - 100 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 20.0 |

* System of scoring:- agglutination partial at serum dilution 1 in 10, 1 mark; complete at 1 in 10, 2 marks; partial at 1 in 20, 3 marks; complete at 1 in 20, 4 marks; partial at 1 in 40, 5 marks; complete at 1 in 40, 6 marks; partial at 1 in 80, 7 marks; complete at 1 in 80, 8 marks; partial at 1 in 160, 9 marks; complete at 1 in 160, 10 marks.

Table 33 shows some fluctuation in the scores in older animals, but the number of animals involved is small in the older age groups. For comparison a similar score was calculated for normal adult cattle, using data obtained from the slaughterhouse samples, the average score being found to be 14.6.

(b) Normal sheep

Blood samples from 330 normal sheep were subjected to agglutination tests with heated antigens, the results being given in Table 34.

TABLE 34

Agglutination titres of 330 samples of normal sheep serum taken at slaughter in tests against heated suspensions of five strains of A. lignieresi

| Strains tested as agglutinable suspension | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|
| | | <10 | 10-20 | 40-160 | 320 | >320 |
| A2 | 0 | 234 | 76 | 20(0) | 0 | 0 |
| A3 | 5 | 155 | 71 | 80(0) | 11 | 13 |
| A7 | 0 | 55 | 38 | 175(8) | 45 | 17 |
| A13 | 1 | 117 | 110 | 103(1) | 0 | 0 |
| A15 | 0 | 202 | 68 | 59(0) | 1 | 0 |

* See footnote to Table 31.

With antigens A2, A13 and A15, the majority of sera gave agglutination at dilutions no higher than 1 in 20 and, except for 1 serum, none agglutinated these three antigens at a dilution higher than 1 in 160. Antigens A3 and A7, however, were agglutinated at considerably higher serum dilutions, this being particularly noticeable with A7 which was agglutinated by 18.8 per cent. of the sera tested, at dilutions of 1 in 320 or more. Prozones were seen in 6 tests, 5 of them occurring with antigen A3. As with cattle, the extent of the prozones was not great, the highest titre obtained being 1 in 160.

(c) Clinical and slaughterhouse cases of bovine actinobacillosis

During the course of collecting slaughterhouse blood samples, 21 cases of actinobacillosis in cattle were seen, A. lignieresi being isolated from the tongue in 10 cases, from lymph glands of the head in 5, from cheek lesions in 3, from the palate in 2, and from the reticulum in 1. The serum from each of these animals was tested against the five heated antigens and, in addition, heated and formolised antigens prepared with the strain of A. lignieresi isolated from the infected tissues (homologous organism). The results obtained

with these tests are given in Table 35. A prozone was present with one or more antigens with 12 of the sera and in most cases the titre of the serum was greater than 1 in 160. The highest titre obtained was 1 in 2560.

TABLE 35

Agglutination titres of sera from 21 bovine carcasses with actinobacillosis observed at meat inspection, in tests against heated and formolised suspensions of A. lignieresi

| Agglutinable suspension | | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|
| Preparation | Strain | | <10 | 10-20 | 40-160 | 320 | >320 |
| Heated .... | A2 | 7 | 7 | 3 | 6 (2) | 1 | 4 |
| | A3 | 1 | 0 | 0 | 20 (3) | 1 | 0 |
| | A7 | 0 | 4 | 9 | 8 (0) | 0 | 0 |
| | A13 | 7 | 4 | 7 | 4 (1) | 1 | 5 |
| | A15 | 7 | 6 | 5 | 3 (1) | 2 | 5 |
| Heated .... | Homologous strain | 5 | 4 | 2 | 4 (2) | 1 | 10 |
| Formolised. | Homologous strain | 2 | 5 | 2 | 6 (2) | 0 | 8 |

* See footnote to Table 31.

Of the 7 sera giving a prozone with antigen A2, one had a titre less than 1 in 160 and two had a titre

of 1 in 160. With antigen A13, one of the 7 sera showing prozoning had a titre less than 1 in 160. In all other cases the sera showing prozones had titres greater than 1 in 160. Seven of these sera from animals known to be infected with A. lignieresi gave completely negative results; their titres fell within the values obtained with normal cattle even in tests with antigens prepared from the infecting organism. When the critical titre was taken as 1 in 640, the agglutination tests with the five standard antigens detected only 6 (28.6 per cent.) of the infected animals. If tests with the antigens prepared from the infecting organism were also considered the number of infected animals detected increased to 12 (57.2 per cent.). When the critical titre was taken as only 1 in 320, the number of cases detected in tests with the five standard antigens rose to 9 (42.9 per cent.) and the number detected in tests with the homologous antigens rose to 14 (66.7 per cent.).

With the cooperation of several practising veterinary surgeons, blood samples and lingual swabs were obtained from 173 cases of clinical actinobacillosis of the tongue. The swabs were cultured on antibiotic blood agar but, in every case,

failed to yield organisms identifiable as A. lignieresi. This result could have been due to a low survival rate of the organism, since the swabs in every instance had been 2 or 3 days in transit through the post. To assess the survival rate of the organism on swabs, a number of "positive" swabs were prepared and subjected to similar conditions of storage to those from clinical cases of actinobacillosis, viz. standing at room temperature and transmission through the post. Two groups of positive swabs were used, one prepared by dipping sterile swabs in nutrient broth cultures of four strains of A. lignieresi and the other by taking up oral mucus from a bovine animal on to swabs and then dipping the swabs into nutrient broth cultures. Immediately the swabs were prepared they were inoculated on antibiotic blood agar and this was repeated 3 days later after the swabs had been sent through the post or had lain at room temperature. On the plates prepared immediately, colonies of A. lignieresi were easily recognisable and were isolated for identification. On the plates prepared from the swabs after transmission through the post A. lignieresi was not detected; the swabs of nutrient broth cultures were now sterile, and those with oral

mucus showed numerous contaminating organisms but no actinobacilli.

The serum samples from the 173 cases of clinical actinobacillosis were examined in agglutination tests with both heated and formolised antigens and the results are set out in Table 36. Sixty-nine sera produced prozones with one or more antigens and most of these sera had titres greater than 1 in 160. The highest titre, found in two sera, was 1 in 20,480 (this was confirmed by a separate titration with the serum dilutions taken to 1 in 81,920). The distribution of the titres of those sera showing prozones is given in Table 37.

TABLE 37

Agglutination titres of sera from clinical cases of actinobacillosis showing prozones

| Agglutinable suspension | | No. of sera showing titre of | | | No. of sera examined |
|---|---|---|---|---|---|
| Preparation | Strain | <160 | 160 | >160 | |
| Heated... | A2 | 0 | 0 | 41 | 41 |
| | A3 | 2 | 0 | 1 | 3 |
| | A7 | 0 | 1 | 1 | 2 |
| Formolised... | A2 | 0 | 1 | 58 | 59 |
| | A3 | 0 | 0 | 1 | 1 |
| | A7 | 0 | 0 | 3 | 3 |
| | A13 | 0 | 0 | 4 | 4 |

TABLE 36

**Agglutination titres of sera from 173 cattle with clinical actinobacillosis in tests against heated and formolised suspensions of five strains of *A. lignieresi***

| Agglutinable suspension | | Total no. of sera examined | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|---|
| Preparation | Strain | | | <10 | 10-20 | 40-160 | 320 | >320 |
| Heated .... | A2 | 173 | 41 | 31 | 42 | 25 (1) | 13 | 62 |
| | A3 | 173 | 3 | 39 | 38 | 73 (9) | 12 | 11 |
| | A7 | 173 | 2 | 20 | 44 | 102 (11) | 4 | 3 |
| | A13 | 170 | 0 | 21 | 73 | 72 (2) | 2 | 2 |
| | A15 | 156 | 0 | 34 | 30 | 87 (4) | 3 | 2 |
| Formolised.. | A2 | 156 | 59 | 19 | 41 | 22 (0) | 7 | 69 |
| | A3 | 144 | 1 | 57 | 49 | 30 (3) | 5 | 3 |
| | A7 | 137 | 3 | 3 | 20 | 96 (16) | 9 | 9 |
| | A13 | 144 | 4 | 9 | 25 | 53 (5) | 20 | 37 |
| | A15 | 16 | 0 | 2 | 6 | 8 (0) | 0 | 0 |

* See footnote to Table 31.

Table 36 shows that a considerable number of sera from cases of actinobacillosis produced agglutination at titres higher than were obtained with normal sera. Not all sera were tested with both heated and formolised antigens, but of the 156 that were examined in this way, 68 (43.6 per cent.) gave completely negative results with titres falling within the values obtained with normal cattle when the critical titre was taken as 1 in 640. Sixty-one of the remaining sera (39.1 per cent.) agglutinated one or more of the heated and formolised antigens at or above this critical titre; 8 (5.1 per cent.) gave a positive result only with the heated antigens and 19 (12.2 per cent.) were positive only with the formolised one. When the critical titre was taken as 1 in 320, 99 (63.5 per cent.) of the sera gave positive results and 57 (36.5 per cent.) gave titres that were within the normal range for cattle. Of the positive sera at this critical level, 75 (48.1 per cent.) agglutinated one or more of both types of antigen to a high titre; 11 (7.1 per cent.) gave a high titre only with the heated antigen and 13 (8.33 per cent.) did so only with the formolised antigen.

## 4. DISCUSSION

Few records are available of the occurrence of antibodies to A. lignieresi in the serum of normal bovine animals. Thompson (1933a) examined the serum from one normal animal with suspensions of six strains of A. lignieresi and obtained titres of 1 in 20 or 1 in 40. Davies (1955, p. 310) stated that serum from healthy animals never agglutinates at a dilution of 1 in 20 and Hebeler et al. (1961) found that the sera of most of their control animals agglutinated one strain of A. lignieresi at dilutions of 1 in 16 or 1 in 32. The present results suggest that these values are low and that titres up to 1 in 160 may be considered normal. Such titres are particularly likely to be obtained when certain strains of A. lignieresi (e.g. A3), which appear to be more highly agglutinable than others, are used as antigen. Although none of the slaughterhouse samples was taken from an animal showing gross lesions of actinobacillosis, minor lesions may have been overlooked during the course of meat inspection and this might account for the three "healthy" animals showing a titre of 1 in 320.

Antibodies to A. lignieresi in the sera of normal sheep have been recorded. Magnusson (1929) noted

titres of 1 in 20 to 1 in 100 in such animals, whilst Taylor (1944) found that 14 normal sheep had serum agglutination titres between 1 in 20 and 1 in 40. As with the findings in cattle, the present results for sheep suggest that previous figures have been low and that considerably higher titres may be expected. It is of particular interest that the two antigenic strains (A3 and A7) that showed agglutination with serum dilutions of 1 in 320 or over, represent antigenic types 2 and 4 into which fell most of the sheep strains isolated from pathological material (Table 9, p. 81). Moreover, antigenic type 4 is one which gave considerable cross agglutination with the actinobacilli isolated from normal animals. No samples of blood from known infected sheep were available for comparison with the "normal" samples so that it is know known whether the high titres which were found represent the normal range for sheep or whether they represent infected animals.

The occurrence of antibodies to A. lignieresi in the sera of cattle and sheep is to be expected in view of the occurrence of actinobacilli in the rumens of normal cattle and sheep and on the tongues of cattle. If A. lignieresi is involved in the process of

microbial digestion in the rumen it is likely that some time will elapse before a population of commensal actinobacilli establishes itself in the rumen of a young animal and, therefore, before any antigenic stimulus can reach the antibody-forming tissues. The gradual appearance of antibodies to A. lignieresi in the sera of young cattle is in agreement with this hypothesis. A similar effect was observed by Tunnicliff (1941) in young normal sheep when two serum titrations were made at the ages of 9 and 31 months. In all cases, except one, there had been an increase in titre.

A striking feature of the results obtained with the sera from clinical and slaughterhouse cases of actinobacillosis was the occurrence of prozones in tests with many of the sera, including some whose agglutinin titre was rather low. This suggests that the development of a prozone in a diagnostic test would be strongly indicative of the presence of infection.

The failure of the agglutination test to detect 43.6 per cent. of the clinical cases of actinobacillosis might be due to the presence in the sample of wrongly diagnosed cases or to cases caused

by antigenic types of A. lignieresi other than those employed as antigens. Although the antigenic strains used in this investigation represent antigenic types 1, 2, 4, 5 and 6 which embrace the majority of the strains derived from pathological material, it is not known whether these are typical of A. lignieresi strains over the whole of Britain, since the geographical distribution of the places of origin of the animals from which the strains from pathological material were obtained was unknown. These animals were examined in the local abattoir, which handles cattle from Scotland and Ireland. It is possible that different antigenic types of actinobacillus occur in other parts of this country and elsewhere in the world.

The short survival time of A. lignieresi on artificially infected mouth swabs supports the findings of Till and Palmer (1960) who showed that the organism was not recoverable later than 5 days after artificial infection of hay and straw. This is in keeping with the low viability of the organism in stock cultures already noted, and lends weight to the view of these workers that A. lignieresi is unlikely to be a soil organism.

## Part V: The relationship of *Actinobacillus lignieresi* with *Bacterium equirulis*

## 1. INTRODUCTION

*Bacterium equirulis* has been given a number of synonyms (Edwards, 1931) including *Bacillus nephritidis equi*, *Bact. viscosum equi* and *Shigella equuli*. More recently it was included in the genus *Achromobacter* as *Achromo. equuli* (Cowan and Steel, 1961), and Breed *et al*. (1957) considered it to fall within the genus *Actinobacillus* and named it *A. equuli*. It is obvious that the taxonomic position of this organism is in some doubt, but the possibility of its relationship with *A. lignieresi* prompted a closer examination of it and comparison with *A. lignieresi*.

*Bact. equirulis* has been recognised as a pathogen of horses and has been isolated from cases of joint-ill in foals (M'Fadyean and Edwards, 1919; Magnusson, 1919; Dimock, Edwards and Bruner, 1947; Maguire, 1958) and from a condition in foals known as "sleepy foal disease" (Report, 1949). The organism has also been associated with disease in pigs. Magnusson (1931) reported the occurrence of arthritis, nephritis and endocarditis in suckling pigs and Ashford and Shirlaw (1962) found endocarditis in a 6-week-old piglet. Post-parturient septicaemia in the sow has been described by Edwards and Taylor (1941),

Bact. equirulis having been isolated in pure culture. Recently a peracute fatal disease caused by infection of piglets with this organism was described by Zimmermann (1964).

Bact. equirulis has been shown to be present in normal horses in the intestinal tract (Laudien, 1923), in the mouth (Cottew and Francis, 1954) and in the tonsillar region (Jarmai, 1929; Dimock et al., 1947).

The purpose of this section of the work is to describe the morphological, cultural and biochemical characters of 8 strains of Bact. equirulis and to investigate their antigenic relationship with A. lignieresi.

## 2. MATERIALS AND METHODS

### (a) Sources of strains of Bacterium equirulis

Seven strains of Bact. equirulis obtained from the National Collection of Type Cultures were employed. These were strains no. 3365, 8529, 8644, 8794, 8795, 8987 and 9435 which were supplied named as Achromobacter equuli. In addition a further strain isolated from a case of verrucose endocarditis in a piglet and reported by Ashford and Shirlaw (1962) was used and given the designation "Shirlaw".

### (b) Media and biochemical reactions

Media employed in this section of the work were the same as those used in Parts I and II (pp. 18 and 70). Biochemical tests, including examination for starch-production were carried out by the same methods as in Part I (pp. 20-25).

### (c) Examination for capsulation

Each strain was examined for the presence of capsules by the method of Duguid (1951). The morphology of the organisms on a number of media was examined in smears stained by Gram's method.

(d) Preparation of antisera

Antisera against heated suspensions of four strains of Bact. equirulis (3365, 8529, 8644 and Shirlaw) were prepared in rabbits in the manner described in Part II (p. 72).

(e) Preparation of antigens

Suspensions of heated bacterial cells for use in slide agglutination tests were prepared as in Part II (p. 72).

## 3. RESULTS

### (a) Morphological and cultural characters

All 8 strains of Bact. equirulis were Gram-negative bacilli, but a marked degree of pleomorphism was apparent in all the smears examined (Fig. 12). In all the strains granules similar to those observed in A. lignieresi were present and "Morse code" forms were seen. The variation in morphology associated with the type of medium on which the organism was grown, which was seen in A. lignieresi cultures, was also observed with Bact. equirulis. Cultures grown on blood agar or nutrient agar showed a majority of short bacillary and coccobacillary forms (Figs. 12 and 13), but on dextrose agar and maltose agar much longer, almost filamentous, forms were seen (Figs. 14 and 15). The breakdown of the filamentous forms into granules with the streptococcal appearance seen with A. lignieresi was also noted with Bact. equirulis (Fig. 14).

In no case were capsules demonstrable in cultures of Bact. equirulis grown on dextrose agar, but evidence of extracellular slime was present. This material was also visible in many of the Gram-stained

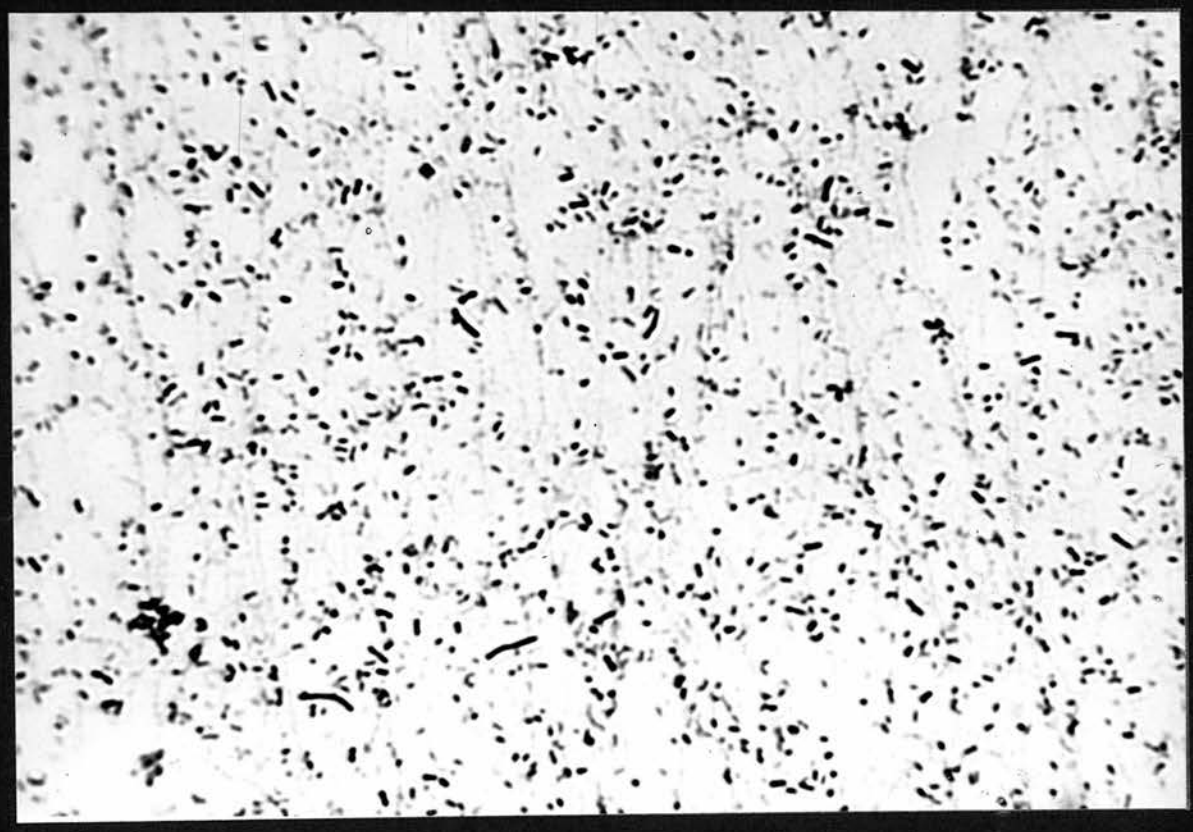

Fig. 12: 24-hour blood agar culture of Bact. equirulis (strain N.C.T.C. 8529) showing coccobacillary and bacillary forms with granules. Faintly staining extracellular slime is present. Gram. X2000.

Fig. 13: 24-hour nutrient agar culture of Bact. equirulis (strain N.C.T.C. 3365) showing coccobacillary forms. Gram. X2000.


Fig. 14: 24-hour dextrose agar culture of Bact. equirulis (strain N.C.T.C. 3365) showing long bacillary and filamentous forms. One filamentous organism shows a granule (a) at one end, and another organism (b) has broken down into bacillary and granular forms. Gram. X2000.

Fig. 15: 24-hour maltose agar culture of Bact. equirulis (strain N.C.T.C. 3365) showing a filamentous organism and long bacilli. Gram. X2000.

smears examined in the form of a faintly staining interstitial substance (Fig. 12).

The colonies of Bact. equirulis on blood agar and nutrient agar were extremely viscous in the case of all except 1 of the strains (N.C.T.C. 3365) and difficulty was always experienced in removing the colonies with an inoculating loop. The viscous nature of the growth was seen also in nutrient broth cultures and the medium increased in viscosity so much that a strand of sticky material was drawn out between the inoculating loop and the surface of the medium when attempts were made to remove a loopful of the culture.

(b) Biochemical characters

Every strain of Bact. equirulis fermented dextrose, laevulose, xylose, sucrose, trehalose and raffinose within 24 hours without gas production, but failed to ferment inulin, dulcitol, salicin and inositol within 14 days. With the remaining substrates there were variations in the reactions obtained and these for the individual strains are recorded in Appendix E and a summary given in Table 38.

Although all strains fermented mannose, maltose, lactose, dextrin and mannitol, several of them were slow to do so.

TABLE 38

Biochemical reactions of Bacterium equirulis

| Test | No. of strains | |
|---|---|---|
| | Positive | Negative |
| Fermentation of:- | | |
| Mannose | 8 (1*) | 0 |
| Galactose | 7 (6*) | 1 |
| Arabinose | 7 (5*) | 1 |
| Rhamnose | 2* | 6 |
| Maltose | 8 (1*) | 0 |
| Lactose | 8 (2*) | 0 |
| Dextrin | 8 (4*) | 0 |
| Glycerol | 6* | 2 |
| Mannitol | 8 (2*) | 0 |
| Catalase production | 4 | 4 |
| Methylene blue reduction | 1+ | 7 |
| Urease test | 7 (1x) | 1 |
| Growth on MacConkey's medium | 6 | 2 |
| Starch synthesis from:- | | |
| Dextrose | 8+ | 0 |
| Maltose | 7+ | 1 |

* Late fermentation (negative at 24 hr; positive within 14 days)
\+ Weakly positive
x Late positive

With the biochemical tests positive results were obtained with all strains in the tests for nitrate reduction and for production of ammonia, and negative results in every case with the V.P. and M.R. tests and those for indole and hydrogen sulphide. Differences between strains with the remaining biochemical tests

are shown in Table 38 and in Appendix E. Failure to grow on MacConkey's medium was seen with two strains. Synthesis of starch from dextrose and maltose was not so active as with A. lignieresi strains and a weak positive reaction was recorded in every case except one negative.

(c) Agglutination reactions

The 4 antisera prepared against strains of Bact. equirulis were tested against heated slide antigens of each of the 8 strains of Bact. equirulis included in this work, as well as against heated slide antigens of A. lignieresi strains. The results with these tests are given in Table 39. Each of the antisera agglutinated Bact. equirulis strains other than the homologous one, but each also agglutinated a number of A. lignieresi strains.

Each of the heated slide antigens of the 8 strains of Bact. equirulis was tested with (a) 27 of the antisera against A. lignieresi strains (antiserum A8 was not included) and (b) the 2 antisera prepared against rumen actinobacilli. Those antisera which agglutinated Bact. equirulis strains are listed in Table 40 (p. 176). Although none of the patterns of

TABLE 39

Agglutination in slide tests of Bact. equirulis and A. lignieresi by 4 antisera against Bact. equirulis strains

| Heated slide antigen prepared with strain | Antiserum prepared against strain:- | | | |
|---|---|---|---|---|
| | 3365 | 8644 | 8529 | Shirlaw |
| Bact. equirulis | | | | |
| 3365 | + | - | - | - |
| 8529 | + | - | + | - |
| 8644 | - | + | - | - |
| 8794 | - | + | - | - |
| 8795 | + | - | + | + |
| 8987 | - | - | - | - |
| 9435 | - | - | - | + |
| Shirlaw | - | - | - | + |
| A. lignieresi | | | | |
| A3 | + | - | - | + |
| A5 | + | - | - | + |
| A6 | - | - | - | - |
| A7 | + | + | - | - |
| A13 | - | - | - | - |
| A14 | - | - | - | - |
| A15 | + | - | + | - |
| A33 | - | + | - | - |
| A43 | - | - | - | - |
| A52 | - | - | - | - |
| A53 | - | + | - | - |
| A75 | - | - | - | - |

TABLE 40

Agglutination in slide tests of Bact. equirulis strains by antisera against 27 A. lignieresi strains and 2 rumen actinobacilli

| Bact. equirulis strain | Agglutinated by antisera |
|---|---|
| 3365 | A49, A60 |
| 8529 | A49 |
| 8644 | A13, A49 |
| 8794 | A7, A49, A53, A60, 43.4 |
| 8795 | A13, A22, A49, A100 |
| 8987 | A13, A43, A49 |
| 9435 | None |
| Shirlaw | None |

agglutination characteristic of the antigenic types of A. lignieresi was found with Bact. equirulis, all strains except two were agglutinated by one or more A. lignieresi antisera and one strain was also acted upon by antiserum 43.4.

## 4. DISCUSSION

The morphological similarities between Bact. equirulis and A. lignieresi are striking, particularly the development of "Morse code" forms and granules. Filaments and streptococcus-like chains were described by Edwards (1931) in his strains of the organism. Capsules were not observed in the strains of the organism examined by Magnusson (1919) and Edwards (1931). The sticky nature of the colonies of Bact. equirulis has been noted by previous workers (Magnusson, 1919; Edwards, 1931) and Magnusson pointed out the slimy growth obtained in fluid cultures. The sticky growth of Bact. equirulis is more evident than that of A. lignieresi.

Edwards (1931) reported that Bact. equirulis did not remain viable for more than 8-10 days on nutrient agar. Magnusson (1931) found with porcine strains that subcultures had to be made every 4 or 5 days in order to maintain the organism, although previously he had reported that the organisms isolated from horses remained alive at room temperature for 5-6 weeks (Magnusson ,1919). The number of strains (all of which were laboratory strains) examined in the present work did not allow of any conclusions on this point,

but Edwards' strains were newly isolated from horses. The similarities in this characteristic between this organism and A. lignieresi are interesting.

The viscous character of the growth of Bact. equirulis is a feature which has been reported on by most workers (Magnusson, 1919, 1931; Cottew and Francis, 1954) but the occurrence of non-mucoid variants in which this property was absent was described by Edwards (1931).

The fermentation of carbohydrates by Bact. equirulis was considered to be more active than that by A. lignieresi (Vallée et al., 1963) and this is borne out in the present work. The fermentation of dextrose, xylose, sucrose, maltose, lactose and raffinose by all strains examined has been observed by most workers (Magnusson, 1919, 1931; Edwards, 1931; Vallée et al., 1963; Wilson and Miles, 1965, p. 521), but Cottew and Francis (1954) noted some variation with xylose and raffinose. There is agreement by all workers on the non-fermentation of dulcitol. Although most investigators have obtained fermentation of laevulose, galactose and mannitol, Vallée et al. (1963) found small numbers of strains which did not attack these substrates whilst Cottew and Francis

(1954) found that all except 2 of their strains failed to ferment mannitol. Strains that did not ferment dextrin were noted by Edwards (1931) and Cottew and Francis (1954). Such slight variations have also been found in the case of substrates which most strains fail to break down, since Edwards (1931) and Vallée et al. (1963) found inulin-fermenting strains and Edwards (1931) described 4 strains which fermented salicin. Cottew and Francis (1954), however, found that all except 2 of their strains fermented salicin. The fermentation of trehalose by all strains examined in the present work shows a marked difference from A. lignieresi which has no effect upon this substrate. With the exception of Cottew and Francis (1954) no other worker has examined the action of Bact. equirulis on trehalose, but these workers observed a small proportion of strains which failed to ferment it.

The findings in the biochemical tests show considerable conformity with those of other workers. Wilson and Miles (1965, p. 521) have recorded a positive catalase reaction as typical of Bact. equirulis, but the present results suggest that there is some variability in this. Although a positive urease reaction has been obtained with most strains

(Vallée et al., 1963; Wilson and Miles, 1965, p. 521), occasional strains may give a negative result. The failure of all strains of Bact. equirulis to produce hydrogen sulphide was regarded as a differential feature between this organism and A. lignieresi (Vallée et al., 1963) and has been noted by Magnusson (1931) and Wilson and Miles (1965, p. 521).

The results with the tests for the production of starch from dextrose and maltose are of interest since, although the reactions obtained were only weakly positive, they suggest the possibility that Bact. equirulis may be involved in the process of microbial digestion in the intestinal tract of the horse and possibly the pig. The incidence of Bact. equirulis in normal horses appears to be high (Dimock et al., 1947; Cottew and Francis, 1954) and this would be in agreement with this hypothesis; also, the reported occurrence of organisms of the Actinobacillus group in the intestines of normal pigs (Dickinson and Mocquot, 1961) lends further weight.

The serological investigations carried out were merely of a preliminary nature, but the results do suggest an antigenic relationship between Bact. equirulis and A. lignieresi. This conclusion was

also reached by Vallée *et al*. (1963). That there is considerable serological heterogeneity amongst *Bact. equirulis* strains was noted by Edwards (1931 and 1932), and Dimock *et al*. (1947) came to the conclusion that this was too great to allow of the use of a diagnostic serological test.

The work reported in this section is intended only as a preliminary survey of *Bact. equirulis* in comparison with *A. lignieresi*. The strains examined are established laboratory strains and it would be useful to extend the comparison to more recently isolated strains. It would be of interest to examine strains of *Bact. equirulis* from the intestinal tract of normal horses and to attempt the recovery of the organism from the intestine of normal pigs.

## CONCLUSIONS AND SUMMARY

A survey of the literature has shown that Actinobacillus lignieresi has not been well characterised by earlier workers and that establishment of its identity has involved the demonstration of its association with the typical actinomycotic lesion. Because of this, the hypothesis that A. lignieresi exists as a commensal organism in the mouth of normal cattle and sheep, although supported by the circumstantial evidence that the incidence of actinobacillosis is associated with factors causing trauma of the oral mucous membrane, has not been confirmed by the isolation of the commensal form of the organism from normal cattle or sheep.

This work has shown that A. lignieresi isolated from pathological material is a Gram-negative bacillus showing marked variation in morphology, depending upon the culture medium. Characteristic granules have been demonstrated which, in association with the bacilli, confer upon cultures a distinctive arrangement that has been named the "Morse code" form.

Actinobacillus lignieresi brings about rapid fermentation of dextrose, laevulose, mannose, xylose, maltose, dextrin and mannitol, but does not attack

trehalose, inulin, dulcitol, salicin and inositol. Fermentation of lactose usually occurs after 6 days, and sucrose is attacked by most strains within 24 hours. Galactose is fermented by all strains, and arabinose and glycerol by most of them, but raffinose is attacked less often whilst rhamnose undergoes change only with occasional strains.

The organism grows on MacConkey's medium, reduces nitrates to nitrites, but fails to produce indole and ammonia. The majority of strains are catalase negative but do produce hydrogen sulphide.

An hitherto unrecorded character of A. lignieresi that has been demonstrated is the ability of many strains to synthesise starch from dextrose and maltose.

The colonies of A. lignieresi are viscous on primary isolation, a character which may be lost on repeated subcultivation. This property is linked with the formation of extracellular slime by A. lignieresi strains, but no capsules are produced. The extracellular slime is antigenic and is heat labile.

Antigenic studies have shown that all except 15 of the strains can be divided into 6 types (no. 1-6) and two subtypes (1a, 4a) on the basis of their

heat-stable antigens. The major antigens concerned in this division are quite distinct, but minor antigens exist which give cross agglutination reactions. In the strains examined those of types 1, 5 and 6 and subtype 1a are all of bovine origin, whilst those of types 3 and 4 are all from sheep. The organisms of type 2 and subtype 4a were derived from both host species.

The heat-labile antigens associated with the extracellular slime are common to organisms of different antigenic types. They may bring about complete inagglutinability of living organisms tested with antisera to the heat-stable antigens. This suggests that the heat-labile antigen is situated superficially on the bacterial cell in the form of a submicroscopic capsule ("microcapsule") which is not always complete, since the inagglutinability of the living cells tested with antisera to the heat-stable antigens may not be absolute.

A medium containing oleandomycin (1 µg/ml.) and nystatin (200 units/ml.) in blood agar has been developed for the isolation of actinobacilli from mixed bacterial populations. Using as selective criteria the colonial and morphological ("Morse code"

forms and granules) characters determined for A. lignieresi, samples of ruminal contents from normal cattle and sheep and swabs from normal bovine tongues yielded organisms resembling A. lignieresi that were recovered from the rumens of 10.1 per cent. of the cattle sampled, and 25.5 per cent. of the sheep, and from the tongues of 37.1 per cent. of cattle examined.

The actinobacilli recovered from normal cattle and sheep showed a slightly greater fermentative activity than the strains of A. lignieresi isolated from lesions. It is suggested that actinobacilli, having the ability to synthesise starch, form part of the iodophilic flora of the rumen and may be concerned in microbial digestion in this organ.

Evidence of the existence of antigens common to A. lignieresi and the actinobacilli isolated from normal animals has been demonstrated. Strains of the actinobacilli have been shown to belong to the antigenic types 1, 3 and 4 of A. lignieresi, but the majority of these strains do not fall into the types and subtypes already recognised, although they show cross agglutination with antisera representing some of these. Cross agglutination reactions have been seen between strains of A. lignieresi (mainly of antigenic

type 4) and an antiserum against one of the rumen actinobacilli.

The occurrence of antibodies to 5 antigenic types of A. lignieresi in the sera of normal adult cattle in titres up to 1 in 160 has been demonstrated in an extensive series of samples. Very young calves do not possess such antibodies, but gradually acquire them during their first year of life. These findings help to substantiate the view that actinobacilli constitute part of the normal flora of the rumen. It is suggested that there is a gradual increase in the population of actinobacilli as the animal moves from milk to the adult diet.

Samples of serum taken from sheep show antibodies to A. lignieresi to slightly higher titres than those from cattle.

The use of the agglutination test as a method of diagnosis of clinical actinobacillosis is not completely satisfactory. Most sera from clinically affected cattle and from slaughterhouse cases of the disease have shown higher levels of antibody than normal animals, but in a number of cases the titres fall within the range for normal animals. A notable feature in tests of clinical and slaughterhouse cases

is the occurrence of a prozone. This does not occur in sera from normal cattle.

Investigation of the characters of Bacterium equirulis (Actinobacillus equuli) has shown close similarities with A. lignieresi. The morphological and cultural characters of the two organisms are markedly alike, "Morse code" forms being a feature of Bact. equirulis also. Although the biochemical characters of the two organisms are distinct, the general fermentative patterns are very similar and cross agglutination reactions are demonstrable between the two species using suspensions of A. lignieresi and Bact. equirulis tested with antisera against several strains of both organisms.

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APPENDIX A: Sources and fermentative and biochemical activities of strains of Actinobacillus lignieresi isolated from pathological material.

All organisms included in this appendix gave the reactions listed below with the following substrates and tests:-

| | | | |
|---|---|---|---|
| Dextrose | + | | |
| Laevulose | + | | |
| Mannose | + | | |
| Xylose | + | Methyl Red Test | - |
| Maltose | + | Nitrates reduced to nitrites | + |
| Trehalose | - | Indole | - |
| Inulin | - | Ammonia | - |
| Dextrin | + | Growth on McConkey's medium | + |
| Mannitol | + | | |
| Salicin | - | | |
| Inositol | - | | |

Key:

Fermentable substrates:
+ = fermentation with acid but no gas within 24 hours.
$+_2$, $+_7$ = fermentation after 2, 7 days.
- = no fermentation within 14 days.

Catalase, methylene blue reduction, Voges-Proskauer and starch production tests:
+ = positive reaction
± = weak positive reaction
- = negative reaction

Urease and $H_2S$ production:
+ = production within 24 hours.
$+_2$, $+_7$ = production after 2, 7 days.
- = not produced within 14 days.

**Strains isolated from slaughterhouse material**

| Strain No. | Host | Site of Lesion | Fermentation of:- Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{6}$ | - | - | - | - | ± | - | + | + | - | + |
| A2 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{6}$ | - | $+_{2}$ | - | - | ± | + | + | $+_{2}$ | ± | ± |
| A3 | Ovine | Lung | + | $+_{14}$ | - | + | $+_{6}$ | $+_{7}$ | $+_{4}$ | - | - | ± | + | + | $+_{2}$ | - | + |
| A4 | Bovine | Rumen | + | $+_{4}$ | - | + | $+_{6}$ | - | $+_{4}$ | - | - | - | + | + | + | ± | + |
| A5 | Ovine | Lymph-gland (head) | + | $+_{14}$ | - | + | $+_{8}$ | - | $+_{4}$ | - | - | ± | + | + | + | + | + |
| A6 | Ovine | Lymph-gland | + | $+_{14}$ | - | + | $+_{6}$ | $+_{4}$ | $+_{4}$ | - | - | - | + | + | $+_{2}$ | + | + |
| A7 | Ovine | S/c abscess (shoulder) | + | $+_{14}$ | - | + | $+_{6}$ | $+_{4}$ | $+_{13}$ | - | - | ± | - | + | $+_{2}$ | + | + |
| A8 | Ovine | Lung | + | $+_{2}$ | - | + | $+_{6}$ | $+_{6}$ | $+_{5}$ | - | - | ± | - | + | + | + | + |
| A9 | Ovine | Lung | + | $+_{2}$ | - | + | $+_{6}$ | $+_{3}$ | + | - | - | ± | - | + | $+_{2}$ | + | + |
| A10 | Ovine | Lung | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | - | + | + | $+_{2}$ | ± | + |
| A11 | Bovine | Tongue | + | $+_{4}$ | - | + | $+_{8}$ | - | $+_{4}$ | $+_{2}$ | - | ± | + | + | $+_{2}$ | - | + |
| A12 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{7}$ | - | $+_{5}$ | - | - | ± | + | + | + | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A13 | Bovine | Retropharyngeal Lymph-gland | + | $+_4$ | - | + | $+_7$ | $+_3$ | $+_7$ | + | - | - | - | ± | $+_2$ | ± | + |
| A14 | Ovine | Lymph-gland (head) | + | $+_3$ | - | + | $+_9$ | - | $+_3$ | - | - | ± | - | ± | + | ± | + |
| A15 | Bovine | Retropharyngeal Lymph-gland | + | - | - | + | $+_{14}$ | - | - | - | - | - | - | ± | + | + | + |
| A16 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_4$ | - | - | ± | - | + | + | ± | + |
| A17 | Bovine | Tongue | + | $+_2$ | - | + | $+_4$ | - | - | - | - | ± | - | + | + | ± | ± |
| A18 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_4$ | - | $+_4$ | - | - | ± | - | + | $+_2$ | ± | + |
| A19 | Bovine | Jaw | $+_2$ | $+_8$ | - | + | $+_3$ | - | $+_7$ | - | - | ± | + | - | $+_2$ | ± | ± |
| A20 | Bovine | Submaxillary Lymph-gland | $+_2$ | $+_8$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | - | + | + | ± | + |
| A21 | Bovine | Jaw | $+_2$ | $+_8$ | - | + | $+_7$ | $+_5$ | $+_5$ | - | - | ± | - | - | + | ± | + |
| A22 | Bovine | Retropharyngeal Lymph-gland | + | $+_8$ | - | $+_2$ | $+_7$ | - | $+_7$ | - | - | ± | - | - | + | ± | + |
| A23 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_8$ | - | + | $+_{14}$ | - | $+_5$ | - | - | - | - | + | + | ± | ± |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A24 | Bovine | Retropharyngeal Lymph-gland | $+_{14}$ | $+_{14}$ | - | + | $+_5$ | $+_4$ | - | - | - | ± | + | ± | $+_3$ | + | + |
| A25 | Bovine | Tongue | + | $+_8$ | - | + | $+_7$ | - | $+_5$ | - | - | ± | - | + | $+_2$ | ± | + |
| A26 | Bovine | Retropharyngeal Lymph-gland | + | $+_8$ | - | + | $+_8$ | - | $+_8$ | - | - | ± | - | - | + | ± | + |
| A27 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_8$ | $+_8$ | + | $+_7$ | - | $+_8$ | - | - | ± | - | + | + | ± | + |
| A28 | Bovine | Submaxillary Lymph-gland | + | $+_8$ | - | + | $+_8$ | $+_3$ | $+_8$ | - | - | ± | $+_{10}$ | - | + | + | + |
| A29 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_8$ | - | + | $+_8$ | $+_5$ | $+_7$ | - | - | ± | - | + | + | ± | + |
| A30 | Bovine | Cheek | + | $+_8$ | - | + | $+_7$ | - | $+_5$ | - | - | ± | - | - | + | ± | + |
| A31 | Ovine | Skin (face) | + | $+_8$ | - | + | $+_3$ | $+_7$ | $+_5$ | - | - | ± | - | + | + | + | + |
| A32 | Bovine | Retropharyngeal Lymph-gland | + | $+_8$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | - | + | + | - | + |
| A33 | Ovine | Skin (head) | $+_5$ | $+_{14}$ | - | + | $+_{14}$ | $+_6$ | $+_5$ | - | - | ± | - | - | + | + | + |
| A34 | Bovine | Tongue | + | - | - | $+_3$ | $+_7$ | - | $+_3$ | - | - | ± | - | - | + | - | + |
| A35 | Bovine | Tongue | $+_2$ | $+_{14}$ | - | + | $+_7$ | - | $+_5$ | - | - | ± | - | - | + | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A36 | Bovine | Reticulum | + | $+_8$ | - | + | $+_5$ | - | $+_5$ | - | - | ± | - | + | + | ± | + |
| A37 | Bovine | Submaxillary Lymph-gland | $+_5$ | $+_{14}$ | - | $+_2$ | $+_7$ | - | $+_5$ | - | - | ± | - | ± | + | ± | + |
| A38 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_5$ | - | $+_5$ | - | - | ± | - | - | + | ± | + |
| A39 | Bovine | Tongue | + | + | - | + | $+_5$ | - | $+_{14}$ | - | - | ± | $+_4$ | + | + | ± | + |
| A40 | Bovine | Tongue | + | $+_3$ | - | + | $+_7$ | - | $+_3$ | - | - | ± | - | + | + | ± | - |
| A41 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_5$ | - | $+_4$ | - | - | ± | - | - | $+_2$ | ± | + |
| A42 | Bovine | Tongue | + | $+_5$ | - | + | $+_7$ | - | $+_3$ | - | - | ± | - | ± | + | ± | + |
| A43 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_3$ | $+_3$ | $+_4$ | - | - | ± | $+_8$ | - | $+_3$ | + | + |
| A44 | Bovine | Cheek | + | + | - | + | - | + | - | - | - | ± | - | - | + | + | + |
| A45 | Bovine | Tongue | $+_2$ | - | - | + | $+_8$ | $+_3$ | $+_5$ | - | - | ± | $+_3$ | ± | $+_2$ | ± | + |
| A46 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_8$ | - | $+_4$ | - | - | ± | - | ± | + | ± | + |
| A47 | Bovine | Submaxillary Lymph-gland | + | + | - | + | $+_7$ | $+_7$ | $+_3$ | - | - | ± | - | + | $+_2$ | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A48 | Bovine | Retropharyngeal Lymph-gland | + | $+_2$ | - | + | $+_5$ | - | $+_{14}$ | - | - | ± | - | - | + | + | + |
| A49 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_5$ | $+_3$ | $+_3$ | - | - | ± | - | ± | + | ± | ± |
| A50 | Bovine | Lung | $+_2$ | $+_{14}$ | - | + | $+_{14}$ | $+_3$ | $+_2$ | $+_2$ | + | ± | - | ± | + | - | - |
| A51 | Bovine | Tongue | $+_3$ | $+_{14}$ | - | + | $+_5$ | $+_2$ | $+_4$ | - | - | ± | - | + | $+_4$ | + | - |
| A52 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_4$ | - | - | - | - | ± | - | - | + | + | + |
| A53 | Ovine | Lung | + | $+_{14}$ | - | + | $+_5$ | - | $+_3$ | - | - | ± | $+_8$ | - | + | + | + |
| A54 | Bovine | Retropharyngeal Lymph-gland | + | - | - | + | $+_9$ | - | $+_4$ | - | - | ± | - | ± | + | ± | + |
| A55 | Bovine | Soft palate | $+_5$ | $+_{14}$ | - | + | $+_5$ | + | $+_5$ | - | - | ± | - | + | + | ± | + |
| A56 | Bovine | Tongue | $+_4$ | $+_4$ | - | + | - | - | $+_2$ | - | - | ± | $+_8$ | + | + | ± | + |
| A57 | Bovine | Tongue | + | - | - | + | $+_9$ | $+_4$ | $+_4$ | - | - | ± | - | + | + | + | + |
| A58 | Bovine | Tongue | + | - | - | + | $+_{14}$ | - | $+_4$ | - | - | ± | - | + | + | ± | + |
| A59 | Bovine | Submaxillary Lymph-gland | + | $+_7$ | - | + | $+_4$ | $+_2$ | - | - | - | ± | - | + | $+_4$ | + | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A60 | Bovine | Retropharyngeal Lymph-gland | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | + | + | + | + |
| A61 | Bovine | Tongue | - | $+_{14}$ | - | + | $+_{4}$ | - | $+_{7}$ | - | - | ± | $+_{2}$ | + | + | ± | + |
| A62 | Bovine | Tongue | $+_{2}$ | $+_{7}$ | - | + | $+_{7}$ | - | $+_{2}$ | - | - | ± | - | - | + | + | + |
| A63 | Bovine | Tongue | + | $+_{7}$ | - | + | $+_{4}$ | - | $+_{4}$ | - | - | ± | - | ± | + | ± | + |
| A64 | Bovine | Tongue | $+_{14}$ | $+_{3}$ | - | + | $+_{14}$ | - | $+_{14}$ | - | - | ± | - | + | + | - | + |
| A65 | Bovine | Reticulum | + | $+_{14}$ | - | + | $+_{7}$ | - | $+_{3}$ | - | - | ± | - | - | + | ± | + |
| A66 | Bovine | Tongue | $+_{14}$ | $+_{2}$ | - | + | $+_{14}$ | - | - | - | - | - | $+_{9}$ | - | + | ± | + |
| A67 | Bovine | Tongue | + | $+_{14}$ | - | + | - | - | $+_{4}$ | - | - | ± | $+_{5}$ | + | + | ± | + |
| A68 | Bovine | Cheek | + | $+_{7}$ | - | + | $+_{8}$ | - | $+_{3}$ | - | - | ± | - | + | + | ± | + |
| A69 | Bovine | Tongue | + | $+_{7}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | ± | - | + | + | ± | + |
| A70 | Bovine | Submaxillary Lymph-gland | + | - | - | + | $+_{7}$ | - | - | - | - | ± | - | ± | + | + | + |
| A72 | Bovine | Tongue | + | $+_{7}$ | - | + | $+_{7}$ | $+_{3}$ | $+_{3}$ | - | - | ± | - | + | + | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A73 | Bovine | Skeletal Muscle (thigh) | + | $+_7$ | - | + | - | $+_7$ | $+_4$ | - | - | ± | - | + | + | ± | - |
| A74 | Bovine | Lung | + | $+_7$ | - | + | $+_{14}$ | - | $+_2$ | - | - | ± | - | ± | + | + | + |
| A75 | Bovine | Retropharyngeal Lymph-gland | + | $+_2$ | - | + | $+_6$ | $+_4$ | - | - | - | - | $+_4$ | ± | + | + | + |
| A76 | Bovine | Retropharyngeal Lymph-gland | + | $+_7$ | - | + | $+_7$ | + | $+_4$ | - | - | ± | - | + | + | + | + |
| A77 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_4$ | - | - | ± | - | + | + | ± | + |
| A78 | Bovine | Submaxillary Lymph-gland | + | - | - | + | $+_7$ | - | + | - | - | ± | - | ± | $+_3$ | ± | + |
| A79 | Bovine | Submaxillary Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_3$ | - | - | ± | $+_7$ | - | $+_3$ | ± | + |
| A80 | Bovine | Tongue | $+_5$ | $+_{14}$ | - | + | - | - | $+_7$ | - | - | ± | - | + | + | + | - |
| A85 | Bovine | Submaxillary Lymph-gland | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_{14}$ | - | - | ± | - | - | $+_3$ | - | - |
| A86 | Bovine | Tongue | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_{14}$ | - | - | ± | + | ± | + | ± | + |
| A87 | Bovine | Tongue | + | $+_7$ | - | + | $+_7$ | $+_7$ | $+_4$ | - | - | ± | - | + | $+_3$ | + | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A88 | Bovine | Tongue | + | +14 | - | + | +14 | - | +5 | - | - | ± | +7 | + | + | ± | - |
| A89 | Bovine | Reticulum | +2 | +14 | - | + | - | - | +5 | - | - | ± | +2 | + | +3 | + | + |
| A90 | Bovine | Tongue | + | +4 | - | + | +3 | +4 | +4 | - | - | ± | - | ± | + | + | + |
| A91 | Bovine | Tongue | +14 | +14 | - | + | +3 | - | - | - | - | ± | +7 | - | +3 | + | ± |
| A92 | Bovine | Cheek | +3 | +14 | - | + | +14 | - | +14 | - | - | ± | - | + | +4 | + | + |
| A93 | Bovine | Retropharyngeal Lymph-gland | +14 | +14 | - | + | +14 | - | +4 | - | - | ± | +8 | ± | +3 | + | + |
| A94 | Bovine | Reticulum | +2 | +14 | - | + | +3 | - | +14 | - | - | ± | - | - | +2 | ± | + |
| A95 | Bovine | Retropharyngeal Lymph-gland | +14 | +14 | - | + | +14 | - | +2 | - | - | ± | - | + | +4 | - | + |
| A96 | Bovine | Submaxillary Lymph-gland | +4 | +14 | - | + | +14 | - | +4 | - | - | ± | +8 | + | +2 | + | + |
| A98 | Bovine | Retropharyngeal Lymph-gland | + | +14 | - | + | +2 | +3 | +2 | - | - | ± | - | + | +2 | + | - |
| A99 | Bovine | Soft palate | + | +14 | - | + | +14 | - | +14 | - | - | ± | +9 | - | +3 | ± | + |
| A100 | Bovine | Retropharyngeal Lymph-gland | +14 | +14 | - | + | +14 | - | +2 | - | - | ± | - | + | + | + | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A101 | Bovine | Retropharyngeal Lymph-gland | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_{2}$ | - | - | ± | - | - | $+_{2}$ | ± | + |
| A102 | Bovine | Tongue | $+_{4}$ | $+_{14}$ | - | + | $+_{4}$ | - | $+_{2}$ | - | - | - | - | - | + | ± | + |
| A103 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | - | $+_{3}$ | - | - | - | ± | - | - | $+_{3}$ | + | + |
| A104 | Bovine | Tongue | $+_{3}$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_{14}$ | - | - | ± | - | + | $+_{2}$ | ± | + |
| A105 | Bovine | Retropharyngeal Lymph-gland | $+_{2}$ | $+_{14}$ | - | + | - | - | $+_{3}$ | - | - | ± | - | - | $+_{2}$ | + | + |
| A106 | Bovine | Tongue | $+_{2}$ | $+_{14}$ | - | + | $+_{5}$ | - | $+_{2}$ | - | - | ± | $+_{8}$ | - | $+_{2}$ | ± | + |
| A107 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{3}$ | - | - | ± | $+_{8}$ | + | $+_{2}$ | ± | + |
| A108 | Bovine | Tongue | $+_{5}$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_{5}$ | - | - | ± | $+_{3}$ | + | $+_{2}$ | ± | + |
| A109 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{3}$ | - | $+_{3}$ | - | - | ± | $+_{5}$ | + | $+_{2}$ | ± | + |
| A110 | Ovine | Prescapular Lymph-gland | + | $+_{14}$ | - | + | $+_{4}$ | + | $+_{14}$ | - | - | ± | - | + | $+_{2}$ | + | + |
| A111 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{4}$ | $+_{4}$ | - | - | - | ± | - | + | $+_{2}$ | + | + |
| A112 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | - | $+_{4}$ | $+_{3}$ | $+_{14}$ | - | - | ± | - | ± | + | + | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A113 | Bovine | Tongue | + | - | - | + | - | - | - | - | - | ± | $+_9$ | - | + | ± | + |
| A114 | Bovine | Tongue | $+_3$ | $+_{14}$ | - | + | $+_5$ | - | + | - | - | ± | $+_6$ | ± | + | ± | + |
| A115 | Bovine | Tongue | $+_2$ | $+_{14}$ | - | + | $+_7$ | - | $+_{14}$ | - | - | ± | - | + | $+_2$ | ± | + |
| A116 | Bovine | Tongue | $+_{14}$ | $+_{14}$ | - | + | $+_3$ | - | $+_4$ | - | - | ± | $+_7$ | + | $+_3$ | ± | ± |
| A117 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | - | $+_5$ | $+_5$ | - | - | ± | - | + | $+_2$ | + | + |
| A118 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_5$ | - | - | - | - | ± | - | + | $+_2$ | ± | ± |
| A119 | Bovine | Tongue | + | $+_{14}$ | - | + | - | $+_7$ | $+_7$ | - | - | ± | $+_7$ | ± | $+_2$ | + | + |
| A120 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | $+_4$ | - | + | ± | ± |
| A121 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | - | $+_3$ | - | - |
| A122 | Bovine | Tongue | $+_3$ | $+_{14}$ | - | + | $+_7$ | - | - | - | - | ± | $+_2$ | ± | + | ± | + |
| A123 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_7$ | $+_7$ | - | - | - | ± | - | + | $+_3$ | ± | ± |
| A124 | Bovine | Retropharyngeal Lymph-gland | + | - | - | + | - | - | - | - | - | ± | $+_5$ | ± | + | ± | + |
| A125 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_5$ | - | - | ± | - | + | $+_3$ | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A126 | Bovine | Tongue | + | - | - | + | $+_4$ | - | $+_7$ | - | - | ± | $+_2$ | + | $+_3$ | + | + |
| A127 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_7$ | - | - | ± | - | + | + | - | + |
| A128 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | - | - | - | - | - | ± | - | - | + | + | + |
| A129 | Bovine | Retropharyngeal Lymph-gland | $+_4$ | $+_{14}$ | - | + | - | - | - | - | - | ± | $+_5$ | + | + | - | - |
| A130 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | - | ± | + | ± | + |
| A131 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | + | $+_4$ | + | + |
| A132 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_5$ | - | $+_{14}$ | - | - | ± | - | + | + | ± | + |
| A133 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | - | + | $+_3$ | + | + |
| A134 | Bovine | Liver | $+_7$ | $+_{14}$ | - | + | - | - | $+_4$ | - | - | ± | - | + | $+_3$ | - | - |
| A135 | Bovine | Retropharyngeal Lymph-gland | $+_7$ | $+_{14}$ | - | + | - | $+_4$ | $+_7$ | - | - | ± | - | + | $+_3$ | - | + |
| A136 | Bovine | Tongue | + | $+_{14}$ | - | + | + | $+_5$ | - | - | - | ± | - | ± | $+_4$ | + | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A137 | Bovine | Retropharyngeal Lymph-gland | $+_4$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_4$ | - | - | ± | $+_4$ | ± | + | + | ± |
| A138 | Bovine | Submaxillary Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_7$ | - | $+_3$ | - | - | ± | $+_7$ | ± | $+_3$ | + | + |
| A139 | Bovine | Tongue | $+_5$ | $+_{14}$ | - | + | $+_5$ | - | $+_7$ | - | - | ± | - | ± | + | + | + |
| A140 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_5$ | - | $+_7$ | - | - | ± | - | - | + | ± | + |
| A141 | Bovine | Retropharyngeal Lymph-gland | $+_4$ | $+_{14}$ | - | + | $+_2$ | - | $+_7$ | - | - | ± | - | - | $+_5$ | ± | + |
| A142 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_7$ | - | $+_2$ | - | - | ± | - | ± | $+_5$ | ± | + |
| A143 | Bovine | Retropharyngeal Lymph-gland | $+_2$ | $+_{14}$ | - | + | $+_2$ | - | - | - | - | ± | - | - | + | + | + |
| A144 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_3$ | - | - | ± | - | + | $+_5$ | ± | + |
| A145 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_7$ | - | $+_7$ | - | - | ± | $+_3$ | + | + | - | + |
| A146 | Bovine | Retropharyngeal Lymph-gland | $+_4$ | $+_{14}$ | - | + | + | - | - | - | - | ± | $+_3$ | - | $+_5$ | + | + |
| A147 | Bovine | Retropharyngeal Lymph-gland | + | $+_7$ | - | + | $+_{14}$ | - | $+_4$ | - | - | ± | $+_7$ | + | $+_3$ | - | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A148 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{7}$ | - | $+_{7}$ | - | - | ± | - | + | $+_{5}$ | ± | + |
| A149 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{5}$ | - | - | ± | - | + | $+_{5}$ | - | + |
| A150 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{7}$ | - | - | ± | - | + | $+_{2}$ | ± | - |
| A151 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | - | - | $+_{7}$ | - | - | ± | $+_{2}$ | + | + | ± | + |
| A152 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{5}$ | - | - | ± | $+_{2}$ | + | $+_{3}$ | ± | ± |
| A153 | Bovine | Retropharyngeal Lymph-gland | $+_{2}$ | $+_{14}$ | - | + | $+_{7}$ | - | $+_{3}$ | - | - | ± | $+_{7}$ | - | + | + | + |
| A154 | Bovine | Retropharyngeal Lymph-gland | + | $+_{7}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | ± | $+_{2}$ | + | $+_{5}$ | ± | ± |
| A155 | Bovine | Lung | + | $+_{3}$ | - | + | - | - | $+_{4}$ | - | - | ± | $+_{2}$ | + | $+_{2}$ | ± | - |
| A156 | Bovine | Submaxillary Lymph-gland | $+_{2}$ | - | - | + | $+_{14}$ | - | $+_{4}$ | - | + | ± | $+_{3}$ | - | $+_{6}$ | ± | ± |
| A157 | Bovine | Retropharyngeal Lymph-gland | $+_{3}$ | - | - | + | $+_{5}$ | - | $+_{4}$ | - | - | ± | - | + | $+_{3}$ | ± | ± |
| A158 | Bovine | Retropharyngeal Lymph-gland | + | - | - | + | - | - | $+_{14}$ | - | - | ± | $+_{2}$ | + | $+_{5}$ | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A159 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{14}$ | - | - | ± | $+_5$ | + | $+_2$ | - | ± |
| A160 | Bovine | Cheek | + | $+_4$ | - | + | $+_5$ | - | $+_7$ | - | - | ± | $+_2$ | + | + | ± | + |
| A161 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_7$ | - | - | ± | $+_2$ | ± | $+_4$ | ± | + |
| A162 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | - | $+_4$ | $+_5$ | - | - | ± | $+_5$ | ± | - | + | + |
| A163 | Bovine | Jaw | $+_3$ | $+_{14}$ | - | + | - | - | $+_5$ | - | - | ± | - | ± | $+_2$ | ± | + |
| A164 | Bovine | Tongue | $+_2$ | $+_{14}$ | - | + | $+_{14}$ | - | $+_5$ | - | - | ± | $+_5$ | + | $+_4$ | ± | + |
| A165 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | + | $+_3$ | ± | ± |
| A166 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_4$ | - | $+_5$ | - | - | ± | $+_2$ | ± | $+_3$ | ± | + |
| A167 | Bovine | Tongue | $+_2$ | $+_{14}$ | - | + | $+_5$ | - | $+_5$ | - | - | ± | $+_2$ | ± | $+_2$ | - | ± |
| A170 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_5$ | - | $+_7$ | - | - | ± | $+_5$ | ± | $+_2$ | ± | + |
| A171 | Bovine | Retropharyngeal Lymph-gland | $+_3$ | $+_{14}$ | - | + | $+_{14}$ | + | $+_7$ | - | - | ± | - | ± | $+_2$ | ± | + |
| A172 | Bovine | Tongue | $+_4$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | $+_4$ | ± | + |
| A173 | Bovine | Tongue | $+_4$ | + | - | + | $+_{14}$ | - | - | - | - | ± | - | + | $+_4$ | - | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A174 | Ovine | Skin (head) | $+_2$ | $+_7$ | - | + | $+_5$ | $+_7$ | $+_{14}$ | - | - | ± | $+_4$ | + | $+_2$ | + | + |
| A175 | Ovine | Lung | $+_3$ | $+_3$ | - | + | $+_7$ | - | - | - | - | ± | - | + | $+_2$ | ± | ± |
| A178 | Bovine | Tongue | $+_2$ | - | - | + | - | - | - | - | - | ± | - | + | $+_5$ | + | ± |
| A179 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | + | ± | ± |
| A180 | Bovine | Retropharyngeal Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | $+_7$ | - | - | - | ± | - | - | $+_2$ | + | + |
| A181 | Bovine | Tongue | $+_2$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | $+_4$ | ± | ± |
| A182 | Bovine | Retropharyngeal Lymph-gland | $+_4$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | $+_4$ | ± | ± |
| A183 | Bovine | Tongue | $+_3$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | $+_7$ | + | + | - | + |
| A184 | Bovine | Liver | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | $+_7$ | + | + | ± | + |
| A185 | Bovine | Tongue | $+_{14}$ | $+_{14}$ | - | + | $+_{14}$ | $+_5$ | - | - | - | ± | $+_7$ | + | + | ± | + |
| A186 | Bovine | Tongue | + | - | - | + | $+_3$ | - | $+_4$ | - | - | ± | $+_3$ | + | + | ± | - |
| A187 | Bovine | Tongue | + | - | - | + | $+_3$ | - | $+_4$ | - | - | ± | $+_4$ | + | $+_2$ | ± | ± |
| A188 | Bovine | Retropharyngeal Lymph-gland | + | - | - | + | $+_3$ | - | $+_2$ | - | - | ± | $+_4$ | + | + | ± | ± |

| Strain No. | Host | Site of Lesion | Fermentation of:- | | | | | | | | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | | | | | | | |
| A189 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{7}$ | - | $+_{5}$ | - | - | ± | - | + | + | ± | ± |
| A190 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | ± | - | + | $+_{2}$ | + | + |
| A191 | Bovine | Tongue | + | - | - | + | $+_{14}$ | - | - | - | - | ± | - | - | + | - | - |
| A192 | Bovine | Tongue | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | ± | - | ± | - | ± | ± |
| A193 | Bovine | Jaw | + | - | - | + | $+_{14}$ | - | $+_{3}$ | - | - | ± | - | ± | $+_{6}$ | + | + |
| * A195 | Bovine | Submaxillary Lymph-gland | + | - | - | + | $+_{4}$ | - | $+_{5}$ | - | - | ± | $+_{4}$ | + | $+_{3}$ | ± | ± |
| A196 | Bovine | Palate | + | - | - | + | $+_{11}$ | - | $+_{3}$ | - | - | ± | $+_{4}$ | ± | $+_{3}$ | - | - |
| A197 | Bovine | Tongue | + | - | - | + | - | - | - | - | - | ± | $+_{4}$ | ± | - | - | ± |
| A198 | Bovine | Liver | + | $+_{14}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | - | ± | $+_{3}$ | ± | + | ± | - |
| A199 | Bovine | Tongue | + | - | - | + | $+_{3}$ | - | $+_{4}$ | - | - | ± | $+_{3}$ | ± | + | - | + |
| A200 | Ovine | Lung | + | $+_{2}$ | - | + | $+_{14}$ | - | $+_{4}$ | - | + | ± | - | ± | $+_{3}$ | + | + |
| A201 | Ovine | Brain | + | $+_{14}$ | - | + | $+_{7}$ | - | $+_{4}$ | - | - | ± | $+_{4}$ | ± | - | ± | ± |
| A202 | Ovine | Submaxillary Abscess | $+_{4}$ | - | - | + | - | - | $+_{14}$ | - | - | ± | - | + | $+_{2}$ | + | + |
| A203 | Ovine | Submaxillary Abscess | $+_{4}$ | $+_{14}$ | - | + | $+_{7}$ | - | - | - | - | ± | - | + | - | + | + |

**Miscellaneous Strains**

| Strain No. | Host | Site of Lesion | Fermentation of:- Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 132/58 | Ovine | Skin (face) | $+_2$ | $+_{14}$ | - | + | $+_2$ | - | $+_3$ | - | - | ± | $+_5$ | + | + | + | + |
| 79/58 | Ovine | Skin (face) | $+_2$ | $+_{14}$ | - | + | $+_2$ | $+_5$ | $+_5$ | - | - | ± | - | ± | + | + | + |
| 2939/57 | Ovine | Skin (face) | + | - | - | + | $+_4$ | - | $+_7$ | - | - | ± | $+_5$ | ± | + | ± | + |
| 2771/52 | Ovine | Lung | $+_3$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | $+_7$ | + | - | + | + |
| 2560/53 | Bovine | Throat (granuloma) | $+_5$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | + | - | - |
| 493/53 | Ovine | Lung | $+_2$ | $+_{14}$ | - | + | $+_7$ | - | - | - | - | ± | $+_7$ | ± | + | ± | + |
| 2491/53 | Ovine | Skin (head) | $+_2$ | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | + | + | + |
| * A194 | Bovine | Tongue | + | - | - | + | $+_3$ | - | $+_4$ | - | - | ± | $+_4$ | + | $+_3$ | - | - |

**Strains isolated from slaughterhouse material (with blood samples)**

| Strain No. | Host | Site of Lesion | Fermentation of:- Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21/14/4 | Bovine | Tongue | $^{+}5$ | $^{+}14$ | - | + | - | - | - | - | + | ± | - | ± | + | ± | ± |
| 1/18/4 | Bovine | Retropharyngeal Lymph-gland | $^{+}4$ | $^{+}14$ | - | + | - | - | $^{+}9$ | - | + | ± | - | ± | + | ± | + |
| 1/12/5 | Bovine | Tongue | + | $^{+}14$ | - | + | - | $^{+}2$ | $^{+}7$ | - | - | ± | - | ± | + | + | + |
| 1/14/5 | Bovine | Mediastinal Lymph-gland | $^{+}7$ | $^{+}14$ | - | + | - | - | - | - | - | ± | - | ± | + | + | + |
| 2/14/5 | Bovine | Tongue | $^{+}7$ | $^{+}14$ | - | + | - | - | $^{+}6$ | - | + | ± | - | ± | + | + | + |
| 1/16/5 | Bovine | Tongue | $^{+}2$ | $^{+}14$ | - | + | $^{+}8$ | - | $^{+}11$ | - | + | ± | - | ± | + | ± | + |
| 21/23/5 | Bovine | Cheek | $^{+}7$ | $^{+}14$ | - | + | $^{+}4$ | $^{+}11$ | - | - | + | ± | - | ± | + | ± | + |
| 1/26/5 | Bovine | Tongue | $^{+}14$ | $^{+}14$ | - | + | - | $^{+}8$ | - | - | - | ± | - | ± | + | + | + |
| 1/31/5 | Bovine | Lymph-gland | $^{+}2$ | $^{+}14$ | - | + | - | - | - | - | + | ± | - | + | + | ± | + |
| 1/3/6 | Bovine | Reticulum | $^{+}2$ | $^{+}14$ | - | + | $^{+}5$ | - | - | - | + | ± | - | ± | + | ± | + |
| 2/3/6 | Bovine | Palate | $^{+}2$ | $^{+}14$ | - | + | $^{+}6$ | - | - | - | + | ± | - | + | + | ± | + |
| 3/3/6 | Bovine | Palate | $^{+}2$ | $^{+}14$ | - | + | $^{+}6$ | - | - | - | - | ± | - | ± | + | ± | + |
| 1/10/6 | Bovine | Cheek | $^{+}4$ | $^{+}14$ | - | + | - | - | $^{+}7$ | - | - | ± | - | ± | + | ± | + |

| Strain No. | Host | Site of Lesion | Fermentation of:- Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2/10/6 | Bovine | Cheek | +6 | +14 | - | + | +5 | - | +14 | - | + | ± | - | + | + | ± | ± |
| 2/12/6 | Bovine | Tongue | + | +14 | - | + | +7 | - | - | - | - | ± | - | ± | + | ± | + |
| 1/13/6 | Bovine | Tongue | +3 | +14 | - | + | - | - | - | - | - | ± | +12 | ± | + | ± | + |
| 1/26/6 | Bovine | Lymph-gland | +14 | +14 | - | + | +14 | +5 | +5 | - | - | ± | - | ± | + | ± | + |
| 1/2/7 | Bovine | Tongue | +3 | +14 | - | + | +5 | - | - | - | - | ± | - | ± | + | ± | + |
| 1/6/8 | Bovine | Lymph-gland | + | +14 | - | + | - | +3 | - | - | - | ± | +3 | - | + | + | + |
| 1/27/8 | Bovine | Tongue | +4 | +14 | - | + | - | - | +6 | - | - | - | - | ± | +2 | ± | + |

| National Collection of Type Cultures Strains | | | Fermentation of:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | Host | Site of Lesion | Galactose | Arabinose | Rhamnose | Sucrose | Lactose | Raffinose | Glycerol | Sorbitol | Catalase | Methylene Blue Redn. | Urease | Voges-Proskauer Test | $H_2S$ Prodn. | Starch from Dextrose | Starch from Maltose |
| 4975 | Bovine | "Typical bovine lesions" | + | - | - | + | - | - | - | - | - | ± | $+_9$ | + | - | ± | + |
| 4976 | Bovine | "Typical bovine lesions" | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | + | $+_7$ | ± | + |
| 4189 | Bovine | Submaxillary Lymph-gland | $+_{14}$ | $+_{14}$ | - | + | - | + | - | - | - | ± | - | + | $+_6$ | ± | + |
| 4191 | Bovine | Submaxillary Lymph-gland | + | $+_{14}$ | - | + | $+_{14}$ | - | - | - | - | ± | - | ± | $+_3$ | ± | + |
| 4985 | Unknown | Unknown | + | $+_{14}$ | - | + | - | - | - | - | - | ± | - | ± | $+_3$ | ± | + |

APPENDIX B: Fermentative and biochemical activities of strains of Actinobacilli isolated from normal animals.

All organisms included in this appendix gave the reactions listed below with the following substrates and tests:-

| | | | |
|---|---|---|---|
| Dextrose | + | | |
| Laevulose | + | | |
| Sucrose | + | Methyl Red Test | - |
| Maltose | + | Nitrates reduced to nitrites | + |
| Trehalose | - | Indole | - |
| Inulin | - | Ammonia | - |
| Mannitol | + | Growth on McConkey's medium | + |
| Dulcitol | - | | |
| Salicin | - | | |

Key:

Fermentable substrates: + = fermentation with acid but no gas within 24 hours.
$+_2$, $+_7$ = fermentation after 2, 7 days.
- = no fermentation within 14 days.

Catalase, methylene blue reduction, Voges-Proskauer and starch production tests: + = positive reaction; ± = weak positive reaction; - = negative reaction

Urease and $H_2S$ production: + = production within 24 hours
$+_2$, $+_7$ = production after 2, 7 days
- = not produced within 14 days

(a) Strains isolated from normal bovine tongues

| Strain No. | Fermentation of:- Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M6.2 | - | $+_3$ | + | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | + | $+_3$ | + | ± | - | ± | $+_6$ | - | - |
| M7.6 | $+_{14}$ | $+_{14}$ | + | + | - | - | - | $+_3$ | $+_{14}$ | + | $+_3$ | + | ± | - | ± | + | ± | + |
| M10.1 | - | $+_3$ | + | + | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_3$ | - | + | - | + | ± | - | ± | $+_6$ | ± | + |
| M12.1 | - | - | + | + | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_3$ | - | + | $+_3$ | + | ± | - | ± | + | + | - |
| M14.1 | - | + | + | + | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_3$ | $+_{14}$ | + | $+_{14}$ | + | ± | - | ± | + | - | ± |
| M17.2 | - | $+_{14}$ | $+_{14}$ | + | - | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | + | $+_{14}$ | + | ± | - | ± | + | ± | ± |
| M20.1 | $+_{14}$ | $+_7$ | $+_{14}$ | $+_2$ | - | $+_7$ | $+_{14}$ | $+_2$ | $+_7$ | + | $+_{14}$ | + | ± | - | ± | + | - | - |
| M21.2 | $+_2$ | $+_2$ | + | + | + | $+_7$ | $+_7$ | $+_2$ | $+_2$ | + | - | + | ± | - | ± | + | ± | - |
| M22.1 | + | $+_7$ | + | + | - | $+_7$ | $+_7$ | $+_2$ | $+_2$ | + | - | + | ± | - | ± | + | ± | ± |
| M23.8 | $+_{14}$ | $+_7$ | + | + | - | $+_7$ | $+_7$ | $+_2$ | $+_7$ | + | - | + | ± | - | ± | $+_4$ | - | ± |
| M24.3 | $+_{14}$ | $+_7$ | + | + | + | $+_7$ | - | $+_2$ | $+_7$ | + | $+_{14}$ | + | ± | - | ± | + | ± | ± |
| M29.2 | $+_{14}$ | + | + | + | $+_{14}$ | $+_5$ | $+_{14}$ | + | $+_{14}$ | + | $+_{14}$ | + | ± | - | ± | $+_2$ | + | - |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| M35.8 | +14 | +9 | + | + | - | +7 | +14 | + | +14 | + | - | | | + | ± | - | ± | +2 | ± | ± |
| M40.6 | +9 | + | +14 | + | - | +2 | +14 | + | +9 | + | - | | | + | ± | - | ± | +2 | - | - |
| M41.7 | - | +9 | +9 | + | - | +7 | - | + | +9 | + | - | | | - | ± | - | ± | +2 | - | - |
| M45.7 | +14 | +9 | +9 | +6 | - | +7 | +9 | + | +9 | + | +7 | | | + | ± | - | ± | +2 | - | ± |
| M46.1 | +14 | + | +9 | + | - | +10 | - | + | +9 | + | - | | | - | ± | - | ± | +2 | ± | - |
| M48.1 | +14 | +9 | - | + | - | +7 | +14 | +6 | +14 | + | +7 | | | - | ± | - | ± | +2 | - | - |
| M51.2 | + | + | - | + | + | + | - | + | +6 | + | +7 | | | + | - | - | - | +3 | - | ± |
| M60.2 | +14 | +3 | - | + | - | +14 | +14 | + | +14 | + | +4 | | | + | - | - | - | +2 | - | - |
| M61.2 | +14 | +3 | + | + | - | +4 | +14 | + | +3 | + | - | | | + | ± | - | ± | +2 | - | + |
| M62.5 | + | +14 | +14 | + | - | +14 | +14 | + | - | + | +4 | | | + | ± | - | ± | +2 | - | ± |
| M63.5 | +7 | +3 | + | + | - | +4 | - | + | +14 | + | - | | | + | ± | - | ± | +2 | - | ± |
| M66.1 | +7 | +3 | +3 | +3 | - | +14 | +14 | + | +14 | + | - | | | + | ± | - | ± | +2 | ± | - |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| M67.1 | + | $+_3$ | + | + | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_3$ | $+_{14}$ | + | - | | | + | ± | - | - | $+_2$ | - | + |
| M75.2 | + | + | + | + | - | - | - | $+_2$ | $+_{14}$ | + | $+_{14}$ | | | + | ± | - | - | $+_2$ | ± | + |
| M76.6 | $+_7$ | $+_{14}$ | + | + | - | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | + | - | | | + | ± | - | - | $+_2$ | ± | + |
| M78.6 | $+_{14}$ | - | + | + | - | $+_{14}$ | $+_{14}$ | $+_3$ | $+_{14}$ | + | - | | | + | ± | - | - | $+_2$ | - | ± |
| M79.4 | $+_7$ | $+_{14}$ | $+_{14}$ | + | - | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | + | - | | | + | ± | - | ± | $+_5$ | ± | + |
| M82.7 | $+_3$ | $+_3$ | $+_{14}$ | + | - | $+_4$ | $+_{14}$ | + | $+_3$ | + | - | | | + | ± | - | - | $+_3$ | - | - |
| M86.3 | + | + | + | + | - | $+_{15}$ | $+_4$ | + | $+_{14}$ | + | $+_3$ | | | + | ± | - | ± | $+_5$ | - | - |
| M91.7 | + | + | + | + | $+_{14}$ | $+_{15}$ | $+_{14}$ | + | $+_{14}$ | + | - | | | + | ± | - | ± | $+_2$ | - | - |
| M93.7 | + | + | + | + | - | $+_{15}$ | $+_{14}$ | + | $+_2$ | + | - | | | + | ± | - | ± | $+_3$ | - | - |
| M94.6 | + | + | + | + | - | - | - | $+_{14}$ | $+_{14}$ | + | - | | | + | ± | - | ± | $+_2$ | - | - |
| M95.6 | + | + | + | + | $+_{14}$ | $+_{15}$ | $+_{14}$ | + | $+_{14}$ | + | - | | | + | ± | - | ± | $+_3$ | - | - |
| M98.1 | $+_7$ | + | + | + | + | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | $+_{14}$ | - | | | + | ± | - | ± | $+_2$ | - | + |

| Strain No. | Fermentation of: | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
| M99.5 | $+_7$ | $+_{14}$ | + | + | - | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_4$ | + | $+_{14}$ | + | ± | - | - | $+_2$ | - | + |
| M101.2 | + | + | + | + | - | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | + | - | + | ± | - | - | $+_2$ | - | - |
| M102.7 | $+_7$ | + | + | + | - | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | $+_{14}$ | - | + | ± | - | - | $+_2$ | - | + |

(b) Strains isolated from ruminal contents of normal cattle

| Strain No. | Fermentation of:- Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.5 | + | + | $+_7$ | + | - | $+_7$ | - | + | $+_7$ | - | - | - | ± | - | ± | $+_3$ | ± | + |
| 19.2 | - | $+_7$ | + | + | - | $+_7$ | $+_4$ | + | $+_7$ | + | $+_5$ | - | ± | - | - | $+_3$ | + | + |
| 37.5 | $+_7$ | $+_6$ | $+_7$ | $+_7$ | $+_7$ | $+_7$ | $+_6$ | $+_7$ | $+_7$ | + | - | + | ± | - | ± | + | - | ± |
| 39.4 | $+_7$ | $+_7$ | $+_7$ | $+_7$ | - | - | $+_5$ | $+_7$ | - | + | - | + | ± | - | ± | + | + | ± |
| 43.4 | - | $+_2$ | $+_2$ | $+_7$ | - | $+_7$ | - | $+_7$ | $+_3$ | + | - | - | ± | - | ± | + | - | ± |
| 57.3 | - | $+_5$ | $+_{14}$ | + | $+_{14}$ | $+_{10}$ | $+_{11}$ | + | $+_{11}$ | - | - | + | ± | - | ± | $+_3$ | - | - |
| 86.4 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | + | $+_8$ | $+_{14}$ | + | $+_2$ | + | - | + | ± | - | ± | + | ± | + |
| 97.3 | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_7$ | - | $+_{11}$ | $+_{14}$ | + | $+_7$ | $+_{14}$ | - | + | ± | - | ± | + | - | - |
| 112.5 | - | $+_7$ | $+_{14}$ | + | $+_{14}$ | $+_7$ | $+_7$ | + | + | + | - | + | ± | - | ± | $+_4$ | ± | ± |
| 117.2 | $+_7$ | $+_3$ | $+_{14}$ | + | $+_{14}$ | $+_7$ | $+_7$ | + | $+_7$ | + | - | + | ± | - | ± | $+_4$ | - | - |
| 31B1 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | $+_9$ | $+_9$ | + | $+_{14}$ | + | - | + | ± | - | ± | + | - | + |
| 82B4 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | - | $+_{14}$ | $+_6$ | + | $+_6$ | + | - | + | ± | - | ± | + | - | ± |

| Strain No. | Fermentation of:- Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 86B2 | $+_{14}$ | $+_{5}$ | $+_{14}$ | + | $+_{14}$ | $+_{14}$ | $+_{5}$ | + | $+_{5}$ | + | - | + | ± | - | ± | + | + | ± |
| 89B3 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | $+_{14}$ | $+_{6}$ | $+_{7}$ | $+_{14}$ | + | - | + | ± | - | ± | + | + | + |
| 95B8 | $+_{14}$ | $+_{7}$ | $+_{14}$ | + | - | $+_{7}$ | - | + | $+_{5}$ | + | - | + | ± | - | ± | + | - | ± |
| 97B3 | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | + | $+_{6}$ | + | - | + | ± | - | ± | + | - | - |
| 98B2 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | $+_{7}$ | $+_{14}$ | + | $+_{14}$ | + | - | + | ± | - | ± | + | - | ± |
| 108B3 | $+_{14}$ | - | $+_{14}$ | + | - | $+_{6}$ | - | + | $+_{14}$ | + | - | + | ± | - | ± | $+_{2}$ | - | - |
| 113B8 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | $+_{3}$ | $+_{14}$ | $+_{3}$ | $+_{3}$ | $+_{3}$ | - | + | ± | - | ± | + | - | + |
| 117B8 | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | - | - | $+_{3}$ | $+_{14}$ | - | - | + | ± | - | ± | + | - | - |
| 130B5 | + | $+_{15}$ | $+_{9}$ | $+_{6}$ | + | $+_{6}$ | $+_{15}$ | + | - | + | - | + | ± | - | ± | $+_{3}$ | - | + |
| 132B3 | + | $+_{6}$ | $+_{15}$ | + | - | $+_{6}$ | $+_{15}$ | + | - | + | - | + | ± | - | ± | + | - | - |
| 162B4 | + | + | $+_{14}$ | + | $+_{14}$ | $+_{6}$ | $+_{14}$ | + | $+_{14}$ | + | - | + | ± | - | ± | + | + | + |
| 163B2 | $+_{14}$ | + | + | $+_{14}$ | - | $+_{14}$ | - | $+_{7}$ | $+_{7}$ | + | $+_{7}$ | + | ± | - | ± | + | ± | ± |

| Strain No. | Fermentation of:- | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | |
| 167B6 | + | $+_{14}$ | $+_{14}$ | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | + | ± | - | ± | + | - | - |
| 168B1 | - | $+_{14}$ | + | $+_{14}$ | - | $+_{14}$ | $+_{6}$ | $+_{14}$ | - | + | - | + | ± | - | ± | + | - | + |
| 171B3 | $+_{7}$ | $+_{14}$ | $+_{14}$ | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | $+_{7}$ | $+_{14}$ | $+_{7}$ | $+_{14}$ | + | ± | - | ± | + | ± | + |
| 173B3 | $+_{14}$ | + | $+_{14}$ | + | + | $+_{14}$ | + | + | + | - | - | + | ± | - | ± | $+_{5}$ | ± | + |
| 185B2 | $+_{14}$ | $+_{14}$ | + | $+_{14}$ | - | $+_{14}$ | - | + | $+_{9}$ | + | - | + | ± | - | ± | $+_{5}$ | - | + |
| 186B4 | $+_{14}$ | $+_{14}$ | $+_{7}$ | $+_{14}$ | - | $+_{14}$ | - | $+_{7}$ | $+_{7}$ | $+_{7}$ | - | + | ± | - | ± | + | - | + |
| 189B8 | - | $+_{7}$ | $+_{14}$ | $+_{14}$ | - | $+_{14}$ | $+_{14}$ | $+_{7}$ | $+_{5}$ | $+_{7}$ | - | + | ± | - | ± | + | - | - |

(c) Strains isolated from ruminal contents of normal sheep

| Strain No. | Fermentation of:- Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S4/24/4 | - | $+_3$ | + | - | - | - | $+_3$ | + | $+_9$ | + | - | + | + | - | - | | - | - |
| S10/26/4 | $+_7$ | $+_2$ | + | - | - | $+_7$ | - | + | - | + | - | + | + | $+_5$ | + | | ± | + |
| S3/27/4 | $+_7$ | + | + | - | - | $+_4$ | + | - | $+_4$ | + | - | + | + | - | + | | ± | - |
| S4/27/4 | + | + | + | - | $+_{14}$ | $+_2$ | - | + | $+_6$ | + | - | - | + | + | - | | ± | ± |
| S3/1/5 | + | + | + | - | $+_{11}$ | $+_3$ | - | + | $+_{11}$ | + | - | - | + | + | - | | + | + |
| S6/3/5 | + | + | + | + | $+_{14}$ | $+_3$ | - | + | $+_8$ | + | - | - | + | $+_3$ | - | | + | + |
| S9/3/5 | - | $+_2$ | + | $+_2$ | - | $+_3$ | $+_7$ | - | $+_{14}$ | + | - | + | + | - | - | | ± | + |
| S1/4/5 | $+_{14}$ | $+_2$ | + | $+_6$ | + | $+_3$ | $+_5$ | - | $+_2$ | + | $+_6$ | + | + | - | - | | ± | - |
| S4/5/5 | + | + | + | + | - | $+_2$ | - | + | $+_6$ | + | - | - | + | $+_3$ | ± | | + | + |
| S3/5/5 | $+_{14}$ | + | + | + | $+_{14}$ | $+_4$ | - | - | $+_{14}$ | + | $+_{14}$ | + | + | - | ± | | - | - |
| S5/5/5 | - | + | + | + | - | $+_5$ | $+_{14}$ | + | $+_9$ | + | - | + | + | - | - | | - | - |
| S10/5/5 | - | + | + | + | - | $+_3$ | - | - | $+_6$ | + | $+_5$ | + | + | - | - | | - | - |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| S1/8/5 | - | + | + | + | - | $+_4$ | $+_3$ | - | $+_3$ | + | - | | | + | + | - | - | | + | + |
| S5/8/5 | + | + | + | + | - | $+_{14}$ | - | - | $+_4$ | + | - | | | + | + | - | - | | + | - |
| S7/8/5 | $+_4$ | + | + | + | - | $+_{14}$ | $+_3$ | + | $+_{14}$ | + | - | | | + | + | $\pm$ | - | | - | - |
| S10/8/5 | + | + | + | + | - | $+_4$ | - | + | $+_{14}$ | $+_{14}$ | - | | | - | + | $+_3$ | + | | + | + |
| S7/10/5 | $+_{14}$ | + | + | $+_3$ | $+_4$ | $+_{14}$ | $+_9$ | - | $+_9$ | + | - | | | + | + | - | + | | - | $\pm$ |
| S5/11/5 | $+_7$ | + | + | + | - | $+_8$ | $+_7$ | + | $+_7$ | + | $+_8$ | | | + | - | - | - | | + | - |
| S6/11/5 | - | + | + | + | - | - | - | - | $+_{14}$ | + | - | | | + | $\pm$ | - | + | | + | + |
| S7/11/5 | $+_{14}$ | + | + | $+_2$ | $+_{14}$ | $+_4$ | $+_2$ | + | $+_3$ | + | - | | | + | + | - | + | | + | + |
| S9/11/5 | - | + | + | + | - | $+_8$ | $+_{14}$ | + | - | + | $+_4$ | | | + | $\pm$ | - | $\pm$ | | $\pm$ | - |
| S8/12/5 | $+_{14}$ | + | $+_2$ | + | $+_{14}$ | $+_3$ | $+_8$ | + | $+_{14}$ | + | - | | | + | + | - | $\pm$ | | $\pm$ | - |
| S9/15/5 | - | + | + | + | - | $+_7$ | $+_6$ | + | $+_{14}$ | + | $+_{14}$ | | | + | $\pm$ | - | - | | - | - |
| S1/17/5 | + | + | + | + | - | - | - | + | $+_8$ | + | - | | | - | + | + | - | | + | + |

| Strain No. | Fermentation of:- Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S2/17/5 | - | + | + | + | - | $+_4$ | $+_8$ | - | $+_8$ | + | $+_{14}$ | + | + | - | - | | + | + |
| S5/17/5 | - | + | + | + | - | $+_3$ | $+_8$ | + | $+_8$ | + | - | + | + | - | - | | + | + |
| S7/17/5 | $+_{14}$ | + | + | + | - | - | - | + | $+_{14}$ | + | $+_9$ | + | + | - | - | | - | - |
| S7/18/5 | - | + | + | + | - | $+_4$ | $+_8$ | - | - | + | $+_{14}$ | + | + | - | - | | ± | - |
| S10/18/5 | + | + | + | + | - | $+_2$ | $+_8$ | + | - | + | $+_4$ | + | + | + | + | | + | + |
| S5/19/5 | + | + | + | + | - | $+_4$ | - | + | - | + | - | - | + | + | + | | ± | + |
| S6/19/5 | - | + | + | + | - | - | - | - | - | + | - | + | ± | - | - | | ± | - |
| S5/29/5 | + | + | $+_4$ | + | - | $+_5$ | - | + | $+_4$ | + | - | - | + | + | - | | + | + |
| S7/29/5 | + | $+_{14}$ | $+_{14}$ | + | - | $+_7$ | $+_{14}$ | $+_{14}$ | - | + | - | + | + | - | - | | + | + |
| S8/29/5 | - | $+_6$ | + | + | + | $+_7$ | - | + | $+_4$ | + | - | + | + | - | + | | - | + |
| S8/31/5 | - | + | $+_3$ | + | - | $+_4$ | $+_{14}$ | + | - | + | - | + | ± | - | + | | ± | + |
| S1/1/6 | - | + | + | + | - | - | $+_5$ | + | $+_2$ | + | $+_3$ | + | + | - | - | | - | + |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| S2/1/6 | - | + | + | + | - | $+^{3}$ | $+^{5}$ | - | $+^{2}$ | + | - | | | + | + | - | $\pm$ | | + | + |
| S3/1/6 | - | + | + | + | - | $+^{3}$ | $+^{5}$ | + | $+^{14}$ | + | - | | | + | + | - | - | | $\pm$ | + |
| S5/1/6 | + | + | + | + | - | $+^{3}$ | $+^{5}$ | + | $+^{5}$ | + | $+^{3}$ | | | + | + | - | - | | - | - |
| S9/5/6 | + | + | + | + | - | $+^{2}$ | - | + | $+^{14}$ | + | - | | | - | + | - | $\pm$ | | + | + |
| S1/6/6 | - | + | + | + | - | - | - | + | - | + | $+^{5}$ | | | + | + | - | - | | - | - |
| S3/6/6 | $+^{14}$ | + | + | + | - | - | - | + | $+^{14}$ | $+^{14}$ | $+^{14}$ | | | + | $\pm$ | - | $\pm$ | | $\pm$ | $\pm$ |
| S4/6/6 | - | + | $+^{3}$ | + | - | $+^{3}$ | $+^{14}$ | + | - | + | - | | | + | + | - | $\pm$ | | $\pm$ | + |
| S6/6/6 | - | + | + | + | - | - | - | + | $+^{14}$ | + | $+^{5}$ | | | + | + | - | - | | - | - |
| S7/6/6 | - | + | + | + | - | $+^{3}$ | $+^{14}$ | + | $+^{14}$ | + | $+^{3}$ | | | + | + | - | - | | - | - |
| S9/6/6 | $+^{14}$ | + | $+^{3}$ | + | - | - | $+^{3}$ | + | $+^{14}$ | $+^{4}$ | $+^{3}$ | | | + | + | - | - | | - | - |
| S4/8/6 | - | + | + | + | - | $+^{2}$ | $+^{14}$ | + | $+^{14}$ | + | - | | | + | + | - | $\pm$ | | - | - |
| S1/9/6 | - | + | + | + | - | $+^{3}$ | $+^{2}$ | + | $+^{14}$ | $+^{7}$ | $+^{6}$ | | | + | + | - | - | | $\pm$ | $\pm$ |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| S4/9/6 | - | + | + | + | - | $+^{6}$ | - | - | $+^{14}$ | + | $+^{6}$ | | | + | + | - | - | | - | - |
| S1/12/6 | $+^{14}$ | $+^{3}$ | + | + | - | $+^{4}$ | $+^{3}$ | + | $+^{14}$ | + | $+^{4}$ | | | + | + | - | - | | - | - |
| S2/12/6 | - | $+^{3}$ | $+^{3}$ | $+^{3}$ | - | $+^{4}$ | - | + | $+^{4}$ | + | - | | | + | + | - | - | | - | - |
| S3/12/6 | + | + | + | + | - | $+^{2}$ | - | + | $+^{14}$ | + | - | | | - | + | + | - | | $\pm$ | - |
| S5/12/6 | - | + | + | + | - | $+^{4}$ | - | + | - | + | - | | | + | - | - | - | | - | - |
| S7/12/6 | - | + | + | + | - | - | - | + | - | + | - | | | + | + | - | - | | + | - |
| S8/12/6 | - | + | + | + | - | - | - | + | - | + | - | | | + | + | - | - | | + | + |
| S9/12/6 | - | $+^{3}$ | + | + | - | - | - | - | - | + | - | | | + | $\pm$ | - | + | | $\pm$ | - |
| S10/12/6 | - | + | + | + | - | $+^{5}$ | $+^{5}$ | - | - | + | - | | | + | + | - | - | | - | - |
| S1/15/6 | $+^{14}$ | + | $+^{2}$ | + | - | $+^{3}$ | - | - | - | + | - | | | + | + | - | - | | - | - |
| S6/15/6 | $+^{14}$ | + | + | + | - | $+^{3}$ | $+^{14}$ | + | $+^{14}$ | + | $+^{3}$ | | | + | + | - | - | | - | - |
| S7/15/6 | - | + | + | + | - | $+^{3}$ | $+^{14}$ | + | $+^{14}$ | + | $+^{3}$ | | | + | + | - | - | | - | - |

| Strain No. | Fermentation of:- | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | |
| S9/15/6 | +14 | + | + | + | - | +2 | +14 | + | +14 | + | +2 | | + | + | - | - | | + | + |
| S1/19/6 | +14 | + | + | + | - | - | +3 | + | +14 | + | +14 | | + | + | - | - | | - | + |
| S2/19/6 | +14 | + | +3 | + | - | - | +3 | + | +14 | + | +14 | | + | + | - | - | | - | + |
| S3/19/6 | - | + | + | +14 | - | +14 | +14 | - | +14 | + | +14 | | + | + | - | + | | + | - |
| S4/19/6 | +14 | + | + | + | - | - | - | + | +14 | + | +14 | | + | + | - | - | | - | + |
| S9/19/6 | - | + | +3 | + | - | +4 | +14 | - | +14 | + | - | | + | + | - | + | | + | + |
| S1/21/6 | + | + | + | + | - | +3 | - | - | +14 | + | - | | + | + | - | - | | - | + |
| S2/21/6 | - | + | + | + | - | +3 | - | - | - | + | - | | + | + | - | - | | - | + |
| S4/21/6 | +2 | + | + | + | - | +2 | +4 | - | +2 | + | - | | + | + | - | - | | - | + |
| S5/21/6 | - | +2 | + | + | - | - | +2 | + | +14 | + | +14 | | + | + | - | - | | + | + |
| S7/21/6 | - | + | +2 | + | - | +3 | - | - | - | + | - | | + | + | - | + | | + | - |
| S8/21/6 | +2 | + | + | + | - | - | - | + | +14 | + | +3 | | + | + | - | - | | + | + |

| Strain No. | Fermentation of:- | | | | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Voges-Proskauer Test | $H_2S$ Production | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Xylose | Rhamnose | Lactose | Raffinose | Dextrin | Glycerol | Sorbitol | Inositol | | | | | | | | | |
| S1/22/6 | - | + | + | + | - | $+_4$ | - | - | $+_{14}$ | + | $+_4$ | | | + | + | - | - | | + | - |
| S3/22/6 | - | + | + | + | - | $+_{14}$ | $+_{14}$ | - | $+_{14}$ | + | $+_{14}$ | | | + | + | - | - | | + | - |
| S4/22/6 | - | $+_2$ | + | + | - | - | - | + | - | + | - | | | + | + | - | + | | + | + |
| S5/22/6 | - | $+_2$ | + | + | - | $+_{14}$ | - | - | $+_{14}$ | + | $+_{14}$ | | | + | - | - | + | | + | - |
| S6/22/6 | - | $+_2$ | + | + | - | $+_{14}$ | - | - | $+_{14}$ | + | - | | | + | + | - | + | | + | - |
| S8/22/6 | $+_2$ | + | + | + | - | $+_2$ | $+_2$ | - | $+_{14}$ | + | - | | | + | - | - | + | | + | - |
| S10/22/6 | - | + | + | + | - | $+_{14}$ | - | - | $+_{14}$ | + | - | | | + | + | - | + | | + | - |
| S4/23/6 | - | + | + | + | - | - | - | + | - | + | - | | | + | + | - | + | | + | + |
| S5/23/6 | - | + | $+_3$ | + | - | - | - | + | - | + | - | | | + | + | - | + | | + | + |
| S6/23/6 | - | + | $+_3$ | + | - | - | - | + | - | + | - | | | + | + | - | + | | + | + |
| S8/23/6 | - | + | $+_3$ | $+_3$ | - | - | - | + | - | + | - | | | + | - | - | + | | + | + |
| S10/23/6 | - | + | $+_3$ | + | - | - | - | - | - | + | - | | | + | + | - | + | | + | - |

APPENDIX C: **Results of slide agglutination tests with actinobacilli isolated from pathological material and from normal animals.**

Key:

+ = agglutination

- = no agglutination

The reactions for any one strain are given on two consecutive pages.

(a) strains of Actinobacillus lignieresi isolated from pathological material.

| Strain No. | Antiserum against strain no:- A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A2 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - |
| A3 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | + |
| A4 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A5 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| A6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| A7 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A8 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A9 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A10 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A11 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A12 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + |
| A15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(a) strains of Actinobacillus lignieresi isolated from pathological material.

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 1 |
| A2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A3 | - | - | - | - | + | - | - | - | - | - | + | - | - | - | 2 |
| A4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A5 | - | - | - | - | + | - | - | - | - | - | + | - | - | - | 2 |
| A6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| A7 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A8 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A9 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A10 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A11 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A13 | - | - | - | - | + | + | + | + | - | - | + | - | - | - | 5 |
| A14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| A15 | - | - | - | + | - | - | - | - | + | + | - | - | + | - | 6 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A16 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A17 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A18 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A19 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A20 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - |
| A21 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A22 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A23 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A24 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A26 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A27 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A28 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A29 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A30 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A31 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A16 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A17 | - | - | - | + | + | - | - | - | + | + | - | - | - | - | 6 |
| A18 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A19 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A20 | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| A21 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A22 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A23 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A24 | - | - | - | - | - | - | - | - | - | - | + | - | + | - | - |
| A26 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A27 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A28 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | 4a |
| A29 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A31 | + | + | + | + | - | - | - | - | - | + | + | - | - | - | 4 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A32 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A33 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A34 | + | - | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A35 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A36 | + | + | + | - | + | + | - | + | + | + | + | - | - | - | - | - |
| A37 | + | + | + | - | + | + | - | + | + | - | + | - | - | - | - | - |
| A38 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A39 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A40 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A41 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A42 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A43 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A44 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A45 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A46 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A32 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A33 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A34 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1a |
| A35 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A36 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1a |
| A37 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1a |
| A38 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A39 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A40 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A41 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A42 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A43 | - | - | - | - | + | + | + | + | - | - | + | - | - | - | 5 |
| A44 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A45 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A46 | - | - | - | - | + | - | - | - | - | - | - | - | - | - | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A47 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A48 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A49 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A50 | Autoagglutinable | | | | | | | | | | | | | | | |
| A51 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - |
| A52 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A53 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A54 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A55 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A56 | + | + | + | + | + | + | + | - | + | + | + | - | - | - | - | - |
| A57 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| A58 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A59 | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - |
| A60 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A61 | - | - | + | + | + | + | - | + | + | - | - | - | - | - | - | - |

| Strain no. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A47 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A48 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A49 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - |
| A50 | Autoagglutinable | | | | | | | | | | | | | | - |
| A51 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A52 | - | - | - | - | + | + | + | + | - | - | + | - | - | - | 5 |
| A53 | + | + | + | + | + | - | - | - | - | + | + | - | + | - | 4 |
| A54 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1a |
| A55 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A56 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A57 | - | - | - | - | - | - | - | - | + | - | + | - | ... | ... | 2 |
| A58 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A59 | - | - | + | - | + | + | + | + | - | - | + | - | - | - | 5 |
| A60 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| A61 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A62 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A63 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A64 | Autoagglutinable | | | | | | | | | | | | | | | |
| A65 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A66 | + | + | + | + | + | + | - | - | - | + | + | - | - | - | - | - |
| A67 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A68 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - |
| A69 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A70 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A72 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A73 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A74 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A75 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A76 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A77 | + | + | + | + | + | + | + | + | + | + | - | + | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A62 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A63 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A64 | Autoagglutinable | | | | | | | | | | | | | | - |
| A65 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A66 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A67 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A68 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A69 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A70 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A72 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A73 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A74 | - | - | - | + | - | - | - | - | + | - | - | - | ... | ... | 6 |
| A75 | - | - | - | - | + | + | + | + | - | - | + | - | ... | ... | 5 |
| A76 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A77 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A78 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A79 | + | + | + | + | + | + | - | - | + | + | - | - | - | - | - | - |
| A80 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A85 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| A86 | - | - | - | - | - | + | - | - | - | + | - | - | + | + | - | - |
| A87 | + | + | + | - | + | + | - | + | + | + | + | - | - | - | - | - |
| A88 | + | + | + | + | + | + | - | - | + | + | + | - | - | - | - | - |
| A89 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A90 | - | - | - | - | - | - | - | - | - | + | - | - | + | + | - | - |
| A91 | + | + | + | - | + | + | - | + | + | + | + | - | - | - | - | - |
| A92 | + | + | + | - | + | + | - | + | + | + | + | - | - | - | - | - |
| A93 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A94 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A95 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | + |
| A96 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
| A78 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A79 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A80 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A85 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| A86 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| A87 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A88 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A89 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A90 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| A91 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A92 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A93 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A94 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A95 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A96 | - | - | - | - | + | - | - | - | - | - | + | - | ... | ... | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A98 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A99 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - |
| A100 | + | + | + | + | + | - | - | + | + | + | + | - | - | - | - | - |
| A101 | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| A102 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A103 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A104 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A105 | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - | - |
| A106 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A107 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A108 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A109 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A110 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| A111 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A112 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A98 | - | - | - | - | + | + | + | + | - | - | + | - | ... | ... | 5 |
| A99 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A100 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A101 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |
| A102 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A103 | - | - | - | - | + | + | + | + | - | - | + | - | ... | ... | 5 |
| A104 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A105 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A106 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A107 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A108 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A109 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A110 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| A111 | - | - | - | - | + | + | + | + | - | + | - | - | ... | ... | 5 |
| A112 | - | - | - | - | + | + | + | + | - | + | - | - | ... | ... | 5 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strain No.** | **A1** | **A2** | **A4** | **A19** | **A22** | **A46** | **A47** | **A55** | **A56** | **A60** | **A100** | **A133** | **A3** | **A5** | **A6** | **A14** |
| **A113** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A114** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A115** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A116** | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **A117** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **A118** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A119** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **A120** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A121** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A122** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A123** | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| **A124** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **A125** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A126** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| **A127** | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
| A113 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A114 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A115 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A116 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |
| A117 | - | - | - | + | - | - | - | - | + | + | - | - | ... | ... | 6 |
| A118 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A119 | - | - | - | + | - | - | - | - | + | + | - | - | ... | ... | 6 |
| A120 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A121 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A122 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A123 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A124 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A125 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A126 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A127 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strain No.** | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A128 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A129 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A130 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A131 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A132 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A133 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A134 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A135 | + | + | + | + | + | + | - | - | - | + | + | - | - | - | - | - |
| A136 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A137 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - |
| A138 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A139 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A140 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A141 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A142 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
| A128 | - | - | - | + | - | - | - | - | + | + | - | - | ... | ... | 6 |
| A129 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A130 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A131 | - | - | - | + | - | - | - | - | + | + | - | - | ... | ... | 6 |
| A132 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A133 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A134 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A135 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A136 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A137 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A138 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |
| A139 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A140 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A141 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A142 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A143 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A144 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A145 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A146 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A147 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A148 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A149 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A150 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A151 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A152 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A153 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A154 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A155 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A156 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A157 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain no. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
| A143 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A144 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A145 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A146 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A147 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A148 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A149 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A150 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A151 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A152 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A153 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A154 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A155 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A156 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A157 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A158 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A159 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A160 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A161 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A162 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A163 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A164 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A165 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A166 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A167 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A170 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A171 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A172 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A173 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A174 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A158 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A159 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A160 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A161 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A162 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A163 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A164 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A165 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A166 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A167 | - | - | - | - | - | - | - | - | - | - | - | + | ... | ... | 1 |
| A170 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A171 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |
| A172 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A173 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A174 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A175 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| A178 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A179 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A180 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A181 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A182 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A183 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A184 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A185 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A186 | + | + | + | - | + | + | + | + | + | + | + | - | - | - | - | - |
| A187 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A188 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A189 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A190 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A191 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A175 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| A178 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A179 | + | - | + | + | + | - | - | - | - | + | + | - | ... | ... | 4a |
| A180 | - | - | - | + | + | - | - | - | + | - | - | - | ... | ... | 6 |
| A181 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A182 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A183 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A184 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A185 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A186 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| A187 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A188 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A189 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A190 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A191 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| A192 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A193 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A195 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + |
| A196 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| A197 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| A198 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A199 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| A200 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| A201 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| A202 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| A203 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 132/58 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| 79/58 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| 2939/57 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A192 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A193 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| A195 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | - |
| A196 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A197 | - | - | - | - | + | + | + | + | - | - | + | - | ... | ... | 5 |
| A198 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A199 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| A200 | - | - | - | - | + | - | - | - | - | - | + | - | ... | ... | 2 |
| A201 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 3 |
| A202 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 3 |
| A203 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 3 |
| 132/58 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| 79/58 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 3 |
| 2939/57 | + | + | - | + | - | - | - | - | - | - | - | - | ... | ... | 4a |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| 2771/52 | Autoagglutinable | | | | | | | | | | | | | | | |
| 2560/53 | + | + | + | - | + | + | + | + | + | + | + | - | - | - | - | - |
| 493/53 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - |
| 2491/53 | Autoagglutinable | | | | | | | | | | | | | | | |
| 21/14/4 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 1/18/4 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 1/12/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| 1/14/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2/14/5 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 1/16/5 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 21/23/5 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 1/26/5 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 1/31/5 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 1/3/6 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2771/52 | Autoagglutinable | | | | | | | | | | | | | | - |
| 2560/53 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1a |
| 493/53 | - | - | - | - | - | - | - | - | - | - | + | - | ... | ... | 2 |
| 2491/53 | Autoagglutinable | | | | | | | | | | | | | | - |
| 21/14/4 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/18/4 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/12/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 2 |
| 1/14/5 | - | - | - | + | - | - | - | - | + | + | - | - | ... | ... | 6 |
| 2/14/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/16/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 21/23/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/26/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/31/5 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/3/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| 2/3/6 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 3/3/6 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 1/10/6 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 2/10/6 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 2/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1/13/6 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| 1/26/6 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 1/2/7 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 1/6/8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1/27/8 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - |
| N.C.T.C.4975 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| N.C.T.C.4976 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| N.C.T.C.4189 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| N.C.T.C.4191 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| N.C.T.C.4985 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 | Antigenic Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2/3/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 3/3/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/10/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 2/10/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 2/12/6 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| 1/13/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/26/6 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/2/7 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| 1/6/8 | - | - | - | - | + | + | + | + | - | - | - | - | ... | ... | 5 |
| 1/27/8 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| N.C.T.C.4975 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| N.C.T.C.4976 | - | - | - | - | - | - | - | - | - | - | - | - | ... | ... | 1 |
| N.C.T.C.4189 | + | + | - | + | + | - | - | - | - | - | + | - | + | - | 4a |
| N.C.T.C.4191 | - | + | + | + | - | - | - | - | - | - | - | - | ... | ... | 4a |
| N.C.T.C.4985 | + | - | + | + | - | - | - | - | - | - | - | - | ... | ... | 4a |

(b) strains of actinobacilli isolated from normal bovine tongues

| Strain No. | Antiserum against strain no:- A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M6.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M7.6 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| M10.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M12.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M14.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M17.2 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| M20.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M21.2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| M22.1 | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - |
| M23.8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M24.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M29.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M35.8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M40.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M41.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(b) strains of actinobacilli isolated from normal bovine tongues

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M6.2 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M7.6 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| M10.1 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M12.1 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M14.4 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M17.2 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| M20.1 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M21.2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| M22.1 | + | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| M23.8 | - | ... | - | + | + | - | - | - | - | - | + | - | + | + |
| M24.3 | - | ... | - | - | + | - | - | - | - | - | - | - | + | + |
| M29.2 | - | ... | - | - | - | - | - | - | - | - | - | - | + | - |
| M35.8 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M40.6 | - | ... | - | - | + | - | - | - | - | - | - | - | + | - |
| M41.7 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| M45.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M46.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M48.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M51.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M60.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M61.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M62.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M63.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M66.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M67.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M75.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M76.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M78.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M79.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M82.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| M45.7 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M46.1 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M48.1 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M51.2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M60.2 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M61.2 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M62.5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M63.5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M66.1 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M67.1 | - | ... | - | - | - | - | - | - | - | - | + | - | - | - |
| M75.2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M76.6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M78.6 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M79.4 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M82.7 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| M86.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M91.7 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| M93.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M94.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M95.6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| M98.1 | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - | - |
| M99.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M101.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M102.7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| M86.3 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| M91.7 | - | ... | + | + | + | - | - | - | - | - | + | - | - | - |
| M93.7 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M94.6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| M95.6 | - | ... | - | + | + | - | - | - | - | - | - | - | + | - |
| M98.1 | + | ... | + | + | + | - | - | - | - | - | + | - | - | - |
| M99.5 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| M101.2 | - | ... | - | - | - | - | - | - | - | - | - | - | + | + |
| M102.7 | - | ... | - | - | + | - | - | - | - | - | - | - | + | + |

(c) strains of actinobacilli isolated from ruminal contents of normal cattle

| Strain No. | Antiserum against strain no:- A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.5 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| 19.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 37.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39.4 | Autoagglutinable | | | | | | | | | | | | | | | |
| 43.4 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 57.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 86.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 97.3 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 112.5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 117.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 31B1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 82B4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 86B2 | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| 89B3 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 95B8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(c) strains of actinobacilli isolated from ruminal contents of normal cattle

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15.5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 19.2 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| 37.5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 39.4 | Autoagglutinable | | | | | | | | | | | | | |
| 43.4 | - | ... | - | + | + | - | - | - | - | - | + | - | + | + |
| 57.3 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 86.4 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| 97.3 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 112.5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| 117.2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 31B1 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 82B4 | - | ... | - | - | + | - | - | - | - | - | - | - | + | + |
| 86B2 | - | ... | - | - | + | + | - | - | - | - | + | - | - | - |
| 89B3 | - | ... | - | - | - | - | - | - | - | - | + | - | + | + |
| 95B8 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| 97B3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 98B2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 108B3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 113B8 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 117B8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 130B5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 132B3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 162B4 | - | - | + | - | - | - | + | - | - | - | - | - | + | + | + | - |
| 163B2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 167B6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 168B1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 171B3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 173B3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 185B2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 186B4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 189B8 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| 97B3 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 98B2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 108B3 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 113B8 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 117B8 | - | ... | - | - | + | - | - | - | - | - | - | - | + | + |
| 130B5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 132B3 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| 162B4 | + | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| 163B2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 167B6 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 168B1 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| 171B3 | - | ... | - | - | - | - | - | - | - | - | - | + | - | - |
| 173B3 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 185B2 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| 186B4 | - | ... | - | + | + | - | - | - | - | - | - | - | + | + |
| 189B8 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |

(d) strains of actinobacilli isolated from ruminal contents of normal sheep

| Strain No. | Antiserum against strain no:- A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S4/24/4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/26/4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/27/4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/27/4 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| S3/1/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| S6/3/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| S9/3/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/4/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/4/5 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S3/5/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/5/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/5/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/8/5 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S5/8/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/8/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(d) strains of actinobacilli isolated from ruminal contents of normal sheep

| Strain No. | Antiserum against strain no:- A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S4/24/4 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/26/4 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/27/4 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/27/4 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S3/1/5 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S6/3/5 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S9/3/5 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S1/4/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/4/5 | + | ... | + | + | + | - | - | - | - | + | - | - | + | - |
| S3/5/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/5/5 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S10/5/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S1/8/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S5/8/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/8/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| S10/8/5 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S7/10/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/11/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/11/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/11/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/11/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/12/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/15/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/17/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| S2/17/5 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S5/17/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/17/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| S7/18/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/18/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - |
| S5/19/5 | - | - | - | - | - | + | - | - | - | - | - | - | + | + | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| S10/8/5 | + | ... | + | + | + | - | - | - | - | + | - | - | + | - |
| S7/10/5 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S5/11/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S6/11/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/11/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S9/11/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S8/12/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/15/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S1/17/5 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S2/17/5 | - | ... | - | + | + | - | - | - | - | - | - | - | + | - |
| S5/17/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/17/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/18/5 | - | ... | + | - | + | - | - | - | - | - | - | - | - | - |
| S10/18/5 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S5/19/5 | + | ... | - | - | + | - | - | - | - | - | + | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| S6/19/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/29/5 | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - |
| S7/29/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/29/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| S8/31/5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/1/6 | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - |
| S2/1/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/1/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/1/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/5/6 | - | - | - | - | - | - | ± | - | - | - | - | - | - | - | - | - |
| S1/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| S4/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| S6/19/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/29/5 | + | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S7/29/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/29/5 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S8/31/5 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/1/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S2/1/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/1/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/1/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/5/6 | + | ... | + | + | + | - | - | - | - | - | + | - | + | - |
| S1/6/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/6/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/6/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/6/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/6/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| S9/6/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| S4/8/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/9/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/9/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S2/12/6 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S3/12/6 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S5/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/12/6 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S10/12/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/15/6 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/15/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S7/15/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| S9/6/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/8/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S1/9/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/9/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/12/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S2/12/6 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S3/12/6 | + | ... | + | + | + | - | - | - | - | - | + | - | + | - |
| S5/12/6 | - | ... | - | - | + | - | - | - | - | - | + | - | + | - |
| S7/12/6 | - | ... | + | - | - | - | - | - | - | - | - | - | - | - |
| S8/12/6 | - | ... | + | - | - | - | - | - | - | - | + | - | - | - |
| S9/12/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/12/6 | - | ... | - | - | - | - | - | - | - | - | + | - | - | - |
| S1/15/6 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S6/15/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S7/15/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| S9/15/6 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| S1/19/6 | - | - | + | - | - | - | - | - | - | - | - | + | - | - | + | - |
| S2/19/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| S3/19/6 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| S4/19/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/19/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/21/6 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| S2/21/6 | - | + | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| S4/21/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/21/6 | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | + |
| S7/21/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/21/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| S9/15/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S1/19/6 | - | ... | - | - | - | - | - | - | - | - | - | + | - | - |
| S2/19/6 | - | ... | - | - | - | - | - | - | - | - | - | + | - | - |
| S3/19/6 | - | ... | - | + | + | - | - | - | - | - | - | - | + | - |
| S4/19/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S9/19/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/21/6 | - | ... | + | - | + | - | - | - | - | - | - | - | - | - |
| S2/21/6 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S4/21/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/21/6 | - | ... | - | - | - | - | - | - | - | - | + | + | - | - |
| S7/21/6 | - | ... | - | - | + | - | - | - | - | - | + | - | - | - |
| S8/21/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S1/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S3/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strain No.** | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 |
| S5/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/22/6 | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| S10/22/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/23/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S5/23/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/23/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/23/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/23/6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

| | Antiserum against strain no:- | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain No. | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 | 43.4 | 97B3 |
| S5/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S6/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/22/6 | - | ... | - | - | + | - | - | - | - | - | - | - | - | - |
| S10/22/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S4/23/6 | - | ... | - | - | - | - | - | - | - | - | + | - | - | - |
| S5/23/6 | - | ... | + | - | - | - | - | - | - | - | + | - | - | - |
| S6/23/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S8/23/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |
| S10/23/6 | - | ... | - | - | - | - | - | - | - | - | - | - | - | - |

## APPENDIX D: Agglutination titres of sera taken from young cattle at various ages in tests against heated suspensions of five strains of Actinobacillus lignieresi

Titres are expressed as the reciprocals of the highest dilution of serum giving agglutination of the test strain of actinobacillus. "p" before a figure denotes incomplete agglutination of the antigen at that dilution of serum.

Date of sampling:-

| Calf No. H17 | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | 11 | 15 | 19 | 23 | 28 | 32 | 36 | 41 | 46 | 50 | 54 | 59 | 63 | 67 | 72 | 76 | 81 | 87 | 89 | 99 |
| Antigen A2 | 10 | 0 | 0 | p20 | ... | p10 | 0 | 0 | p10 | 10 | 0 | 20 | p20 | p20 | 10 | p40 | p20 | p80 | 40 | p20 |
| " A3 | p10 | 20 | 10 | 0 | ... | p80 | p80 | 80 | p40 | 20 | 20 | 80 | p160 | 160 | p80 | 160 | 80 | 80 | 40 | p80 |
| " A7 | 0 | p10 | p20 | 10 | ... | p10 | 20 | 40 | 20 | p20 | 20 | 20 | 20 | p40 | p40 | p40 | p40 | 80 | p80 | p20 |
| " A13 | 10 | 0 | 20 | 0 | ... | p20 | p80 | 10 | 20 | p10 | 40 | 40 | 40 | 40 | 40 | 20 | 20 | 40 | p80 | p10 |
| " A15 | 10 | 0 | 0 | 0 | ... | 0 | 0 | 0 | 0 | 0 | 0 | p10 | p10 | 0 | p20 | 40 | 20 | p40 | 20 | 40 |
| **Calf No. H18** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | 8 | 12 | 16 | 20 | 25 | 29 | 33 | 38 | 43 | 47 | 51 | 56 | 60 | 64 | 69 | 73 | 78 | 84 | 86 | 96 |
| Antigen A2 | 0 | 0 | 0 | 0 | p10 | 0 | 0 | 0 | p20 | p40 | ... | 20 | p10 | p10 | p10 | 10 | 10 | p20 | p20 | 0 |
| " A3 | 0 | 0 | p10 | p40 | 80 | 80 | p80 | p40 | p80 | 80 | ... | 80 | 80 | 40 | 80 | p160 | 80 | p80 | p20 | 40 |
| " A7 | 0 | 0 | p10 | 0 | 20 | p40 | 10 | p20 | 20 | p40 | ... | 80 | 20 | 20 | 20 | p20 | p40 | p20 | 10 | p20 |
| " A13 | p10 | 0 | 20 | 0 | 0 | 20 | p10 | 0 | p20 | p20 | ... | 20 | 40 | 20 | p20 | p20 | p20 | 20 | p20 | 0 |
| " A15 | p10 | 0 | 0 | p10 | 0 | 0 | 0 | 0 | 0 | 0 | ... | 10 | p10 | p20 | p10 | 40 | 20 | p40 | 20 | 10 |

Date of sampling:-

| Calf No. J1 | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | 5 | 9 | 12 | 17 | 21 | 26 | 30 | 34 | 40 | 44 | 48 | 53 | 57 | 61 | 65 | 70 | 74 | 80 | 83 | 92 |
| Antigen A2 | 0 | p10 | 0 | 0 | 0 | 0 | p10 | p10 | 10 | p10 | 0 | 10 | p20 | 0 | 10 | 20 | 20 | p20 | p20 | p20 |
| " A3 | 0 | 0 | 10 | 20 | 40 | p40 | p40 | 80 | 40 | 80 | 80 | 80 | p160 | 80 | 160 | 160 | 160 | 160 | p160 | p160 |
| " A7 | 0 | 0 | 0 | 0 | 0 | p20 | p10 | 20 | p20 | 0 | p20 | p20 | p10 | p10 | p20 | p10 | 20 | p40 | p40 | p40 |
| " A13 | p10 | 0 | 10 | 0 | p10 | p80 | p20 | 10 | p40 | p10 | p40 | p40 | 20 | p40 | 20 | p10 | p40 | 40 | 20 | 0 |
| " A15 | p10 | p20 | p10 | p10 | p20 | p10 | 0 | p10 | 10 | 0 | 0 | 0 | 40 | p20 | p10 | 20 | p10 | 20 | p40 | 20 |

| Calf No. J2 | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | 1 | 5 | 9 | 14 | 18 | 22 | 26 | 31 | 36 | 40 | 44 | 49 | 53 | 57 | 62 | 66 | 71 | 77 | 79 | 89 |
| Antigen A2 | 0 | 0 | p10 | p10 | 10 | p20 | p10 | p10 | 10 | p20 | p20 | p40 | 20 | p10 | 10 | 20 | 20 | p40 | p20 | 10 |
| " A3 | 0 | 0 | 0 | p40 | 80 | 40 | p80 | 80 | p80 | 40 | 160 | 160 | 160 | p160 | 80 | 160 | 160 | 160 | p160 | p80 |
| " A7 | 0 | 0 | 0 | 0 | p10 | p20 | p20 | 40 | 20 | 20 | 80 | 40 | 20 | 20 | 20 | p40 | 40 | p80 | p20 | 40 |
| " A13 | 0 | 0 | 20 | 0 | 0 | 10 | p20 | 10 | 20 | 20 | p160 | 20 | p20 | p20 | 20 | 20 | p80 | p80 | 20 | 0 |
| " A15 | 0 | p20 | 10 | p10 | p10 | 0 | 0 | 0 | p10 | 0 | p20 | 20 | p20 | 10 | 10 | 20 | p80 | p80 | p40 | 40 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31,3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. J3** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | 1 | 5 | 9 | 14 | 18 | 22 | 26 | 31 | 36 | 40 | 44 | 49 | 53 | 57 | 62 | 66 | 71 | 77 | 79 | 89 |
| Antigen A2 | 0 | 10 | p10 | p20 | 0 | p10 | p10 | p10 | 10 | p20 | 0 | p20 | p20 | p10 | p20 | 20 | 20 | p40 | p20 | 0 |
| " A3 | 0 | 0 | 0 | 20 | 40 | 80 | 80 | 160 | p80 | 160 | 80 | 160 | 160 | 160 | p160 | 160 | 20 | 80 | p160 | 80 |
| " A7 | 0 | 0 | 0 | 0 | p10 | 20 | 10 | 40 | 20 | p20 | 20 | 20 | p40 | 20 | 20 | p40 | p20 | p80 | 20 | p20 |
| " A13 | 0 | 0 | 20 | 0 | p10 | 20 | 10 | 10 | p20 | p10 | p80 | 10 | 20 | p20 | 20 | 20 | 20 | 20 | p40 | 0 |
| " A15 | 0 | 10 | p10 | p10 | 0 | 0 | 0 | p10 | p10 | 10 | 0 | p20 | 20 | 10 | 10 | 20 | 20 | p80 | 40 | 20 |
| **Calf No. J4** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | 1 | 5 | 9 | 13 | 17 | 22 | 26 | 30 | 35 | 40 | 44 | 48 | 53 | 57 | 61 | 66 | 71 | 76 | 79 | 89 |
| Antigen A2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | p10 | 0 | p10 | 0 | 0 | 0 | 10 | 10 | p10 | p10 | 0 |
| " A3 | 0 | 10 | p10 | 20 | 40 | p40 | 20 | 40 | 40 | 20 | 80 | 80 | p80 | p80 | p80 | 80 | p80 | p80 | p40 | p20 |
| " A7 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 20 | 10 | 0 | 20 | p20 | p20 | 10 | 20 | 20 | p20 | 40 | p20 | 20 |
| " A13 | 0 | 0 | 0 | 0 | 0 | p80 | p10 | 0 | p10 | 0 | p10 | p20 | p10 | p10 | 0 | p20 | p20 | 0 | 20 | 0 |
| " A15 | 0 | 0 | 0 | 0 | p10 | 0 | 0 | 0 | p10 | 0 | 0 | p10 | 0 | 0 | p10 | p10 | p40 | 20 | p20 | 20 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. J5** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | 3 | 7 | 12 | 16 | | | | | | | | | | | | | | |
| Antigen A2 | - | - | 0 | p10 | 0 | | | | | | | | | | | | | | | |
| " A3 | - | - | 0 | 0 | p10 | | | | | | | | | | | | | | | |
| " A7 | - | - | 0 | 0 | 0 | Removed from herd. | | | | | | | | | | | | | | |
| " A13 | - | - | 40 | 0 | 0 | | | | | | | | | | | | | | | |
| " A15 | - | - | p10 | 0 | 0 | | | | | | | | | | | | | | | |
| **Calf No. J6** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | 4 | 9 | 13 | 17 | 22 | 27 | 31 | 35 | 40 | 44 | 48 | 53 | 57 | 62 | 68 | 70 | 80 |
| Antigen A2 | ... | ... | ... | 0 | p10 | 0 | 0 | 0 | p10 | 10 | 0 | p20 | p20 | 0 | 10 | p20 | 20 | 20 | 0 | 10 |
| " A3 | ... | ... | ... | 0 | 0 | p20 | 10 | p20 | p20 | 20 | 20 | p40 | p40 | 20 | 40 | 80 | p40 | 80 | p20 | 40 |
| " A7 | ... | ... | ... | 0 | 0 | p20 | 0 | 20 | 20 | p20 | 40 | p40 | 20 | p20 | 20 | 20 | p40 | p20 | p10 | 20 |
| " A13 | ... | ... | ... | 0 | 0 | p20 | 0 | 0 | 10 | 0 | p20 | p10 | p10 | 0 | p20 | 20 | p20 | p20 | p10 | 0 |
| " A15 | ... | ... | ... | 0 | 0 | 10 | p20 | p10 | p10 | 10 | 10 | 0 | p10 | p10 | p10 | 0 | p80 | 20 | p10 | p40 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. J7** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | 3 | 7 | 11 | 15 | 20 | 25 | 29 | 33 | 38 | 42 | 46 | 51 | 55 | 60 | 66 | 68 | 78 |
| Antigen A2 | ... | ... | ... | p20 | 10 | 0 | 0 | p10 | 10 | 10 | 10 | p10 | 20 | p10 | 10 | 10 | 20 | p20 | 0 | 0 |
| " A3 | ... | ... | ... | 0 | 0 | 0 | p10 | 40 | p40 | 80 | 80 | 80 | p160 | 20 | 40 | p80 | p80 | p80 | p20 | 40 |
| " A7 | ... | ... | ... | 0 | 0 | 0 | 0 | p40 | 10 | 20 | p40 | p20 | 20 | p20 | 20 | 20 | p80 | p20 | p20 | 20 |
| " A13 | ... | ... | ... | p10 | 0 | 0 | 0 | 0 | p10 | p40 | 40 | p10 | p20 | p20 | p20 | p20 | 20 | 10 | 0 | 0 |
| " A15 | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p80 | 10 | 0 | 0 | p20 | p10 | 10 | 10 | p80 | p20 | 0 | 10 |
| **Calf No. J8** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | 2 | 7 | 11 | 15 | 20 | 25 | 29 | 33 | 38 | 42 | 46 | 51 | 55 | 60 | 66 | 68 | 78 |
| Antigen A2 | ... | ... | ... | 0 | p10 | 0 | 0 | 0 | p10 | 0 | 0 | p10 | p10 | p10 | p10 | p10 | 20 | p40 | 20 | 0 |
| " A3 | ... | ... | ... | 0 | 0 | 0 | p10 | 10 | p10 | p20 | 20 | 20 | 20 | 20 | p40 | 80 | p80 | p80 | p40 | 20 |
| " A7 | ... | ... | ... | 0 | 0 | 0 | 0 | p10 | p10 | 0 | p10 | p10 | 0 | p20 | 10 | p20 | p40 | p80 | p20 | p10 |
| " A13 | ... | ... | ... | 0 | 0 | 0 | p10 | 0 | 0 | 0 | 0 | p10 | p10 | p20 | 20 | 10 | p40 | p40 | p20 | 0 |
| " A15 | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p10 | 0 | 0 | p10 | p10 | p20 | 10 | p20 | 20 | 20 | 10 | p10 |

| | Date of sampling:- | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
| **Calf No. J9** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | 5 | 10 | 14 | 19 | 23 | 28 | 32 | 36 | 41 | 45 | 49 | 54 | 59 | 64 | 67 | 77 |
| Antigen A2 | ... | ... | ... | ... | 0 | p10 | 0 | 0 | p10 | 0 | 0 | 0 | p10 | 0 | 0 | 10 | 10 | 20 | 20 | 0 |
| " A3 | ... | ... | ... | ... | 0 | 0 | p10 | 20 | p40 | 20 | 160 | 20 | p80 | 20 | 20 | p80 | 80 | 160 | p80 | p160 |
| " A7 | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | 20 | p10 | p10 | p10 | 10 | p20 | p80 | 20 | 10 | p160 |
| " A13 | ... | ... | ... | ... | 0 | 0 | p10 | 0 | p20 | p10 | 160 | 20 | p40 | p20 | 20 | p20 | p40 | 20 | 20 | 0 |
| " A15 | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | p10 | 0 | 0 | 0 | 20 | 40 | 10 | p20 | p80 |
| **Calf No. J10** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | 22 | 26 | 30 | 35 | 39 | 43 | 48 | 52 | 57 | 63 | 65 | 75 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | 10 | 0 | p10 | 10 | p10 | 0 | p10 | p10 | 10 | p20 | p10 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | 20 | p40 | 40 | 80 | p80 | p80 | 40 | 80 | 80 | 80 | 40 | p80 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | 20 | 0 | 20 | 20 | 20 | p40 | 20 | 20 | p20 | 20 | 10 | 0 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p20 | 10 | p40 | p20 | p20 | p20 | 10 | 0 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p10 | p10 | p10 | p10 | 10 | p80 | 80 | p40 | p20 |

| | Date of sampling | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
| **Calf No. J11** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | 1 | 5 | 9 | 14 | 19 | 23 | 27 | 32 | 36 | 40 | 45 | 49 | 54 | 60 | 62 | 72 |
| Antigen A2 | ... | ... | ... | ... | 0 | 0 | p10 | p10 | ... | 20 | p10 | p20 | p20 | p10 | 10 | p10 | 20 | 20 | p10 | 0 |
| " A3 | ... | ... | ... | ... | 0 | 0 | 0 | 20 | ... | p20 | 80 | 80 | p80 | 80 | p80 | p160 | p160 | 160 | p80 | 80 |
| " A7 | ... | ... | ... | ... | 0 | 0 | 0 | p10 | ... | p10 | 20 | p20 | p20 | p10 | p10 | p20 | p20 | 20 | 0 | 20 |
| " A13 | ... | ... | ... | ... | 0 | 0 | 0 | p10 | ... | p10 | 20 | p20 | p10 | p20 | p10 | p20 | p20 | 20 | 0 | 0 |
| " A15 | ... | ... | ... | ... | 0 | 0 | 0 | 0 | ... | 0 | 0 | p20 | p10 | p10 | p20 | p10 | p20 | 40 | 10 | p40 |
| **Calf No. J12** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | 3 | 8 | 12 | 17 | 21 | 26 | 30 | 35 | 38 | 43 | 48 | 52 | 58 | 61 | 70 |
| Antigen A2 | ... | ... | ... | ... | ... | p10 | p10 | p20 | p20 | 20 | p10 | p20 | p10 | 0 | p10 | 10 | p10 | p20 | p40 | 0 |
| " A3 | ... | ... | ... | ... | ... | 0 | 0 | 40 | p20 | p20 | 40 | 80 | 80 | p80 | p40 | 80 | p40 | 40 | p40 | p80 |
| " A7 | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 10 | 20 | p20 | 10 | p20 | p20 | p20 | p40 | p20 | p40 |
| " A13 | ... | ... | ... | ... | ... | 0 | 0 | p10 | 0 | 0 | 40 | 10 | p20 | p20 | p20 | 10 | 10 | 10 | p10 | 0 |
| " A15 | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | p10 | p10 | p20 | 20 | p40 | p20 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. J23** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | 5 | 9 | 14 | 18 | 22 | 27 | 31 | 36 | 42 | 44 | 54 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 20 | p10 | p10 | p20 | 0 | p20 | 20 | p40 | p40 | p20 | p10 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | 20 | 20 | p40 | 80 | p80 | p80 | p20 | p80 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | p10 | p20 | p20 | p20 | 20 | 20 | 10 | p40 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p20 | p20 | p20 | p20 | 20 | 10 | p20 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p10 | 10 | 10 | p20 | 20 | 20 | p40 | 40 |
| **Calf No. J24** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | 1 | 6 | 10 | 14 | 19 | 23 | 28 | 34 | 36 | 46 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | p10 | p10 | 0 | p10 | 10 | p10 | p10 | p10 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p40 | p40 | p40 | p80 | p80 | 80 | p10 | 80 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p10 | 0 | p20 | p10 | p20 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | p10 | p20 | 10 | 0 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. K1** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | - | 1 | 5 | 8 | 13 | 18 | 23 | 28 | 31 | 41 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | p10 | p10 | 0 | 10 | p10 | 10 | 10 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | p20 | p40 | 20 | 20 | p20 | 40 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | 0 | 0 | p20 | p20 | p20 | p40 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p20 | 10 | 10 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | p10 | 0 | p10 | 0 | p10 | 40 | p40 | p40 |
| **Calf No. K2** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | - | - | - | 6 | 11 | 15 | 20 | 26 | 28 | 38 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p20 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 20 | p20 | 20 | p10 | p20 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | p10 | p20 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | p10 | p10 | 0 |

Date of sampling:-

| | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calf No. K3** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | - | - | - | 6 | 11 | 15 | 20 | 26 | 28 | 38 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | p10 | 0 | p20 | 10 | 10 | 0 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | p10 | 40 | p20 | p40 | 0 | 80 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p10 | 0 | p10 | p10 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | p10 | 0 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | p10 | p20 | p10 | 0 |
| **Calf No. K4** | | | | | | | | | | | | | | | | | | | | |
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 | 6 | 11 | 17 | 19 | 29 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | 10 | p40 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p20 | p80 | p20 | 20 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 20 | 10 | p10 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 20 | p20 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 | 0 | 0 |

Date of sampling:-

| Calf No. K5 | 3.2.59 | 3.3.59 | 31.3.59 | 1.5.59 | 1.6.59 | 1.7.59 | 30.7.59 | 1.9.59 | 6.10.59 | 4.11.59 | 4.12.59 | 5.1.60 | 4.2.60 | 1.3.60 | 5.4.60 | 6.5.60 | 9.6.60 | 19.7.60 | 4.8.60 | 12.10.60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (weeks) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 8 | 14 | 16 | 26 |
| Antigen A2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p10 | 0 |
| " A3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p20 | p10 |
| " A7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p20 | 0 |
| " A13 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | p20 | 0 |
| " A15 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0 | 0 | 0 | 0 |

APPENDIX E: Fermentative and biochemical activities of strains of Bacterium equirulis

All organisms included in this appendix gave the reactions listed below with the following substrates and tests:-

| | | | |
|---|---|---|---|
| Dextrose | + | | |
| Laevulose | + | | |
| Xylose | + | Voges-Proskauer test | - |
| Sucrose | + | Methyl Red test | - |
| Trehalose | + | Nitrates reduced to nitrites | + |
| Raffinose | + | Indol | - |
| Inulin | - | Ammonia | + |
| Dulcitol | - | $H_2S$ | - |
| Salicin | - | | |
| Inositol | - | | |

Key:

Fermentable substrates:
+ = fermentation with acid but no gas within 24 hours.
$^{+}_{2}$, $^{+}_{7}$ = fermentation after 2, 7 days.
- = no fermentation within 14 days.

Catalase, methylene blue reduction and starch production tests:
+ = positive reaction.
± = weak positive reaction.
- = negative reaction.

MacConkey's medium:
+ = growth.
- = no growth.

Urease production:
+ = production within 24 hours.
$^{+}_{2}$, $^{+}_{7}$ = production after 2, 7 days.
- = not produced within 14 days.

| Strain No. | Fermentation of:- | | | | | | | | | | Catalase | Methylene Blue Reduction | Urease | Growth on MacConkey's Medium | Starch from Dextrose | Starch from Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mannose | Galactose | Arabinose | Rhamnose | Maltose | Lactose | Dextrin | Glycerol | Mannitol | Sorbitol | | | | | | |
| N.C.T.C. 3365 | $+_5$ | $+_3$ | $+_5$ | - | $+_2$ | $+_2$ | $+_3$ | - | $+_3$ | - | + | ± | - | - | ± | ± |
| N.C.T.C. 8529 | + | $+_5$ | + | $+_6$ | + | + | $+_2$ | $+_5$ | + | - | + | - | + | + | ± | ± |
| N.C.T.C. 8644 | + | - | $+_5$ | - | + | + | + | - | + | + | - | - | + | + | ± | ± |
| N.C.T.C. 8794 | + | $+_{12}$ | - | - | + | + | + | $+_{13}$ | + | + | + | - | + | + | ± | ± |
| N.C.T.C. 8795 | + | $+_2$ | $+_7$ | - | + | + | + | $+_4$ | + | - | - | - | + | + | - | ± |
| N.C.T.C. 8987 | + | $+_3$ | + | - | + | + | + | $+_4$ | + | - | + | - | + | + | ± | ± |
| N.C.T.C. 9435 | + | + | $+_2$ | - | + | + | $+_2$ | $+_4$ | + | - | - | - | + | + | ± | ± |
| Shirlaw | + | $+_2$ | $+_6$ | $+_7$ | + | $+_2$ | $+_2$ | $+_7$ | $+_2$ | + | - | - | $+_2$ | - | ± | ± |

## APPENDIX F: Published papers

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# The Characterisation of *Actinobacillus Lignieresi*

BY

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# THE CHARACTERISATION OF *ACTINOBACILLUS LIGNIERESI*

J. E. PHILLIPS

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(PLATE LIV)

*Actinobacillus lignieresi*, first described by Lignières and Spitz (1902) as the causal agent of disease of cattle, is believed to enter the tissues of cattle and sheep by infection of wounds of the skin and alimentary mucous membrane (Thomas, 1931; Thornton, 1943). It is possible that this organism is a commensal of the alimentary tract (Taylor, 1944), although there is no record of its isolation from normal animals. Examination of the literature, however, suggests that *A. lignieresi* is not well characterised, and that its identity is finally established only by its association with granulomatous lesions in cattle and sheep. This fact would render difficult, if not impossible, confirmation of its commensal role.

This paper describes the morphological, cultural and biochemical characters of 225 strains of *A. lignieresi* isolated from lesions in cattle and sheep.

## MATERIALS AND METHODS

*Media.* Except where otherwise stated the media used in this work were prepared as described by Mackie and McCartney (1948).

*Isolation of organisms from lesions.* Small portions of tissue showing macroscopic lesions of actinobacillosis were inoculated on horse-blood agar plates and glucose broth. Strains of *A. lignieresi* recovered in this way were maintained in cooked-meat medium and each strain was also preserved by drying *in vacuo*. Whenever possible the nature of the lesion was confirmed by histological examination.

*Biochemical reactions.* Each strain was examined for its fermentative activity, production of ammonia, indole, catalase and hydrogen sulphide, reduction of nitrates, hydrolysis of urea and ability to grow on MacConkey's medium. In addition, the methylene blue reduction, methyl red (M.R.) and Voges-Proskauer (V.P.) tests were carried out on each strain.

Fermentative activity was examined in peptone water with Andrade's indicator and the following: dextrose, lævulose, mannose, galactose, arabinose, xylose, rhamnose, sucrose, maltose, lactose, trehalose, raffinose, inulin, dextrin, glycerol, mannitol, dulcitol, sorbitol, salicin and inositol. Incubation was continued for 14 days and readings were made every day.

Five-day-old peptone-water cultures were tested with Nessler's and Ehrlich's rosindol reagents for the presence of ammonia and indole respectively. For the V.P. and M.R. tests, cultures were grown for 4 days in glucose-phosphate-peptone water (Kauffmann, 1954), the Barritt (1936) modification of the V.P. test being used. The nitrate-reduction test was carried out on 4-day-old nitrate-broth cultures (Kauffmann, 1954). Catalase and methylene blue tests

were performed by the methods described by Wilson and Miles (1955, pp. 452 and 453) on 24-hr cultures on nutrient agar slopes and nutrient broth respectively.

*Starch-forming properties.* All strains of *A. lignieresi* were grown overnight on 1 per cent. dextrose and maltose-agar slopes and the cultures then flooded with Gram's iodine diluted 1 in 10.

## Results

Two hundred and twenty-five strains of *A. lignieresi* were examined, of which five were obtained from the National Collection of Type Cultures (no. 4189, 4191, 4975, 4976 and 4985); the remainder were isolated from bovine and ovine tissues and their anatomical and host sources are shown in table I. In 201 cases the lesions presented the typical histological appearance of actinobacillosis.

Table I

*Host-tissue distribution of* Actinobacillus lignieresi *strains*

| Species | No. of strains derived from | | | | Total no. of strains |
|---|---|---|---|---|---|
| | tongue | lung | rumen or reticulum | other tissues | |
| Sheep . . | 0 | 9 | 0 | 15 | 24 |
| Bovine . | 174 | 3 | 6 | 13 | 196 |
| Total . | 174 | 12 | 6 | 28 | 220 |

*Morphological characters.* Although the strains of *A. lignieresi* examined may all be described as Gram-negative bacilli, there was considerable variation in the length of the organisms from one strain to another. In some cases it was decidedly coccobacillary whilst in others the appearance was that of a rather long slender rod. The morphology was found to vary with the medium on which the organism was grown. On nutrient agar, blood agar and Loeffler's serum, the cultures showed a majority of short bacillary or coccobacillary forms, but when the same strain was grown on dextrose or maltose agar much longer forms and even filaments were observed. The intensity of staining by Gram's stain showed considerable variation between individual organisms within the same smear, some bacilli taking the stain well and others being only faintly coloured and having the appearance of " ghost " forms.

A constant feature of cultures of *A. lignieresi* on any medium was the occurrence of small granules scattered amongst the bacilli. These granules stained in the same way as the organisms and in most cases were not attached to the bacilli, although an occasional granule could be seen attached or lying very close to a bacillus (fig. 1). This gave a picture which can be likened to the conventional representation of the letters of the Morse code (e.g. - · and - · - ). Granules of this type were not seen in a large number of other Gram-negative bacteria

*ACTINOBACILLUS LIGNIERESI*

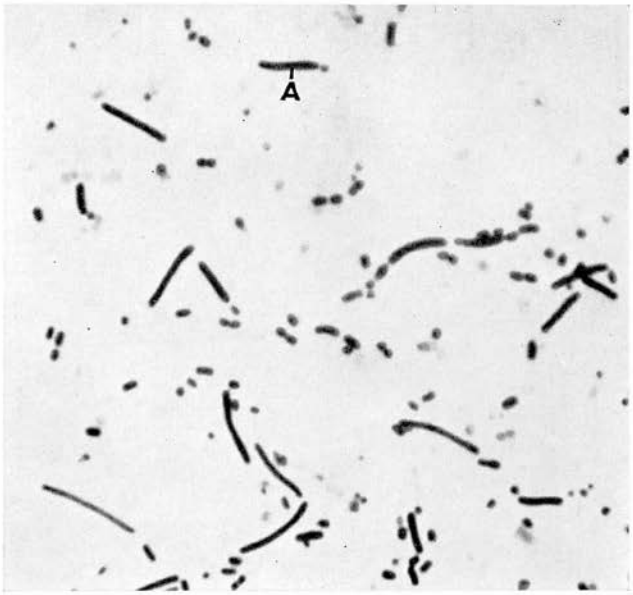


FIG. 1.—24-hr dextrose agar culture of *A. lignieresi* showing long bacilli and a " Morse code " form (A). Gram. ×2400.

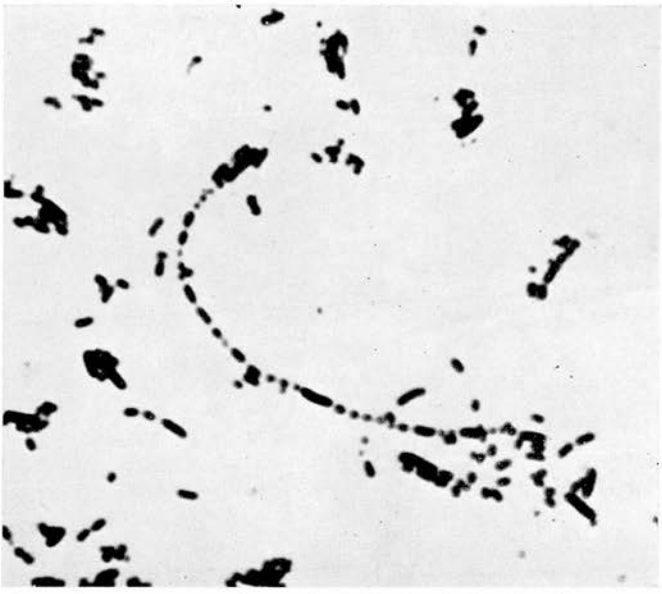

FIG. 2.—24-hour dextrose agar culture of *A. lignieresi* showing filamentous organism broken down into bacillary and granular forms. Gram. ×2800.

examined. The filamentous organisms were occasionally seen to break down into shorter bacillary forms interspersed with granules, giving an almost streptococcal appearance (fig. 2).

*Cultural characters.* Colonies on blood agar were small (1-2 mm. diameter) after 24 hr and gradually increased in size with further incubation. No great variation in colony size was noted, although some strains were found that grew rather less profusely. This character was maintained even after repeated subcultivation. In most cases the colonies were viscous and difficult to remove completely from the surface of the medium, but this feature usually disappeared after several subcultures.

*Biochemical characters.* The 225 strains fermented dextrose, lævulose, mannose, xylose, maltose, dextrin and mannitol within 24 hr

TABLE II

*Fermentation reactions of* A. lignieresi *strains*

| Substrate | Fermentation | |
|---|---|---|
| | positive | negative |
| Lactose . . . | 187 | 38 |
| Sucrose . . . | 222 | 3 * |
| Sorbitol . . . | 3 | 222 |
| Raffinose . . . | 50 | 175 |
| Arabinose . . . | 197 | 28 |
| Glycerol . . . | 163 | 62 |
| Galactose . . . | 127 | 98 * |
| Rhamnose . . . | 1 | 224 |

* Late fermentation.

without the production of gas. No fermentation occurred within 14 days with trehalose, inulin, dulcitol, salicin and inositol. Differences between strains were noted in the reactions with the remaining substrates, and these are shown in table II.

When lactose was fermented, this occurred later than the first 24 hr of incubation, the majority of positive reactions appearing at 5 or 6 days with a range of 2-14 days. Every strain, with the exception of three that did so between the 2nd and 5th days, fermented sucrose promptly. Similarly, all strains fermented galactose eventually, but 43·5 per cent. did so between the 2nd and 14th days. The average times for the fermentation of raffinose, arabinose and glycerol were 4 days, more than 7 days and 5 days respectively.

Neither ammonia nor indole was produced by any of the strains of *A. lignieresi* examined, and the M.R. test was negative in every case. All strains grew on MacConkey's medium and reduced nitrates to nitrites.

The differences between strains with the catalase, V.P., methylene blue, hydrogen sulphide and urease tests are shown in table III. The positive reactions obtained in the methylene blue reduction tests did not go to completion and only a green colour was obtained.

*Starch-forming properties.* Three types of result were obtained in these tests following the addition of iodine to the culture:

(*a*) bacterial growth coloured bluish purple (strong positive);
(*b*) bacterial growth coloured reddish brown (weak positive);
(*c*) bacterial growth not coloured or only slightly yellow (negative).

TABLE III

*Biochemical reactions of* A. lignieresi *strains*

| Test for | Test | |
|---|---|---|
| | positive | negative |
| Catalase production | 12 | 213 |
| V.P. test | 111 | 114 |
| Methylene blue reduction | 215 * | 10 |
| Hydrogen sulphide production | 218 | 7 |
| Urease production | 82 | 143 |

* Reaction incomplete.

The number of strains of *A. lignieresi* producing starch from maltose and from glucose are shown in table IV. No strain of *A. lignieresi* grown on nutrient agar containing no fermentable substrate showed any colour change when iodine was added, so that the reddish brown colour seen in the weak positive strains was not due to staining of the bacterial cytoplasm by the iodine.

TABLE IV

*Starch-forming properties of* A. lignieresi *with two substrates*

| No. of strains giving, with *dextrose* as substrate, test for starch formation | No. of strains giving, with *maltose* as substrate, test for starch formation | | | Totals for strains utilising *dextrose* to synthesise starch |
|---|---|---|---|---|
| | strongly positive | weakly positive | negative | |
| Strongly positive | 64 | 3 | 4 | 71 |
| Weakly positive | 95 | 26 | 7 | 128 |
| Negative | 13 | 4 | 9 | 26 |
| Totals for strains utilising *maltose* to synthesise starch | 172 | 33 | 20 | ... |

## DISCUSSION

The granules observed in cultures of *Actinobacillus lignieresi* appear to be characteristic of this organism, although their existence has not previously been recorded. This feature may be of considerable value in the preliminary screening of organisms from the digestive tract of normal cattle, during the search for the commensal form of *A. lignieresi*.

Most workers who have studied *A. lignieresi* have examined only small numbers of strains and their results have been so variable as to

lead Wilson and Miles (1955, p. 472) to the view that "The fermentative ability of this organism is a little doubtful". Although Tunnicliff (1941) recorded variable results with dextrose, most other workers obtained fermentation without gas formation (Bosworth, 1923; Taylor, 1944; Ristic *et al.*, 1956). Similarly, there is agreement that maltose, mannitol, sucrose, xylose, lævulose, mannose and galactose are fermented, and that dulcitol, inositol, inulin, trehalose, rhamnose and sorbitol are not. One strain of the present series fermented rhamnose and three strains attacked sorbitol; this suggests that fermentation of rhamnose and sorbitol is not a common property of *A. lignieresi*. Jowett (1931), Tunnicliff (1941), Taylor (1944), and others reported that no acid is produced by this organism from salicin, a fact which is substantiated by the present work, although Wramby (1940) obtained variable results. Variable fermentation of dextrin was also reported by Wramby (1940) and by Ristic *et al.* (1956); negative results were reported by Tunnicliff. In the present investigation all strains fermented dextrin, a fact also observed by Taylor (1944). A feature of *A. lignieresi* which has been noted by a number of authors in the past is the late fermentation of lactose (Magnusson, 1929; Jowett, 1931; Vawter, 1933; Taylor, 1944; Wilson and Miles, 1955, p. 472). This has not proved to be a constant feature in this work, since 17 per cent. of strains failed to ferment lactose within 14 days.

The failure of this organism to produce indole confirmed the findings of other workers (Magnusson, 1929; Davies and Torrance, 1930; Taylor, 1944; Ristic *et al.*, 1956), although Wilson and Miles (1955, p. 472) found that indole was formed in small amounts. Growth on MacConkey's medium has been recorded previously only by Hayston (1948). Ristic *et al.* (1956) were the only workers to examine *A. lignieresi* for catalase activity; they found that all of 14 strains gave a positive catalase reaction, with which our findings do not agree. Moreover they found that their strains produced little or no hydrogen sulphide, whereas the present results suggest that the majority of strains are active producers of hydrogen sulphide.

The production of starch by *A. lignieresi* is of interest since, if the organism exists in the commensal state, it may well be that it goes to make up the normal iodophilic flora of the rumen. No direct proof of the commensal hypothesis has yet been provided, chiefly because of the difficulties of identifying the organism accurately in the absence of any specific lesion.

## Summary

*Actinobacillus lignieresi* has been shown to be a Gram-negative bacillus producing characteristic granules. Its primary colonies on blood agar are viscous, but often lose this property after subcultivation. The organism brings about rapid fermentation of dextrose, maltose, mannitol, sucrose, xylose, dextrin, mannose and lævulose, but does not attack dulcitol, inositol, inulin and trehalose. A number of other

substrates may or may not be fermented, but lactose usually gives a late positive reaction after 6 days. *A. lignieresi* grows on MacConkey's medium, reduces nitrates to nitrites and does not form indole.

The ability of this organism to produce starch is described, and it is suggested that it may contribute to the iodophilic flora of the rumen.

I am indebted to Mr J. Norval who provided the slaughterhouse specimens, to Mr R. Hood for the photographs and Dr A. Wilson Taylor for his advice.

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# THE COMMENSAL ROLE OF *ACTINOBACILLUS LIGNIERESI*

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*University of Edinburgh*

The habitat of *Actinobacillus lignieresi*, other than in lesions of actinobacillosis, is not known. It is generally accepted that in actinobacillosis of cattle and sheep, the organism enters the tissues through wounds of the skin and of the oral and ruminal mucous membranes (Thomas, 1931; Thornton, 1943; Gerring, 1947; Hayston, 1948), and it has been suggested that it may exist as a commensal on the skin and in the mouth (Taylor, 1944; Till and Palmer, 1960). Phillips (1960) referred to the difficulties involved in demonstrating the commensal role of this bacterium, but drew attention to a characteristic morphological feature (the "Morse Code" form) which, it was suggested, might prove helpful in identifying the organism in the absence of lesions.

This paper records the recovery from bovine ruminal contents of 31 strains of organisms having morphological, cultural, biochemical and antigenic characters similar to those of *A. lignieresi*.

## MATERIALS AND METHODS

*Media.* The media used in the identification of strains of actinobacillus were those described in a previous paper (Phillips, 1960). Primary isolation of the

TABLE

*Biochemical reactions of rumen actinobacilli*

| Test | No. of strains | |
|---|---|---|
| | positive | negative |
| Fermentation of :— | | |
| Lactose | 29* | 2 |
| Raffinose | 23 (22*) | 8 |
| Glycerol | 27 (25*) | 4 |
| Galactose | 30 (26*) | 1 |
| Rhamnose | 14 (11*) | 17 |
| Inositol | 4* | 27 |
| Mannose | 25 (20*) | 6 |
| Catalase production | 28 | 3 |
| Voges-Proskauer test | 30 | 1 |
| Starch synthesis from :— | | |
| Maltose | 22 (10†) | 9 |
| Glucose | 11 (6†) | 20 |

* Late fermentation ; † weakly positive

organisms from ruminal contents was made on a selective medium consisting of nutrient agar containing 5 per cent. horse blood and 1 μg. oleandomycin and 200 units nystatin per ml.

*Isolation of organisms from ruminal contents.* Antibiotic blood agar plates were inoculated with ruminal material taken from cattle immediately after slaughter at the abattoir. After incubation, likely colonies were picked on to horse blood agar. Organisms having a morphology similar to that of *A. lignieresi* were further examined biochemically.

## Results

As far as could be ascertained in the routine necropsy carried out in the abattoir, none of the 306 animals sampled had lesions of actinobacillosis. The sera of 189 of them were examined serologically for the presence of antibodies against various antigenic types of *A. lignieresi* ; all gave values within the range for normal cattle (Phillips, unpublished).

Actinobacillus-like organisms were recovered from 31 (10 per cent.) of the samples of ruminal contents. All these organisms fermented dextrose, lævulose, arabinose, xylose, sucrose, maltose, dextrin and mannitol without the production of gas, and failed to ferment trehalose, inulin, dulcitol and salicin within 14 days. Negative results were given in every case in the tests for the production of indole, ammonia and urease ; the hydrogen sulphide and methylene blue reduction tests were positive. All strains grew on MacConkey's medium and reduced nitrates to nitrites, but gave negative methyl red tests. The differences observed between strains with other fermentable substrates and biochemical tests are shown in the table.

Several strains were agglutinated by antisera prepared against known pathogenic strains of *A. lignieresi* as well as by an antiserum against one of the rumen organisms. Absorption tests showed a close but not absolute antigenic relationship between one strain of rumen organism and several pathogenic strains (including the National Collection of Type Cultures strain no. 4189).

## Discussion

A feature of the biochemical results was the slightly greater fermentative activity of the organisms recovered from the ruminal contents compared with the previously described strains from lesions (Phillips, 1960), e.g. many fermented raffinose and rhamnose. On the other hand more rumen strains gave *late* fermentation of galactose than was found with pathogenic strains, and many fermented mannose late or not at all. The majority of these strains differ from pathogenic strains in producing catalase (but see Ristic *et al.*, 1956).

The antigenic relationship of the majority of rumen strains with known pathogenic strains of *Actinobacillus lignieresi* suggests that these " normal " actinobacilli may be *A. lignieresi*, and it is suggested that, until their pathogenicity has been assessed and a more thorough antigenic analysis of " normal " actinobacilli and pathogenic strains of *A. lignieresi* has been carried out, the normal strains should be included in this species. The failure of specific antisera to agglutinate some strains suggests that antigenic types other than those recovered from lesions of actinobacillosis may exist. It is possible that there are many antigenic types, of which a few only are pathogenic, but this can be determined only by an extensive survey.

Previously, when the ability of *A. lignieresi* to synthesise starch was recorded, it was suggested that this bacterium might be a constituent of the iodophilic flora of the rumen. If *A. lignieresi* is concerned in microbial digestion in the rumen the slight differences in in-vitro biochemical activities between the " normal " and the pathogenic strains may indicate the more active fermentative role required of the commensal form.

The hypothesis of the commensal nature of *A. lignieresi* in the rumen is consistent with the generally held view that infection with the organism occurs by way of wounds and abrasions, since the most frequent sites of lesions of actinobacillosis are in the mouth, rumen and reticulum and the associated lymph-glands. Hitherto the normal habitat of *A. lignieresi* has been assumed to be the skin or the mouth and tongue (Taylor, 1944 ; Till and Palmer, 1960), although the organism has not been demonstrated in those sites in normal animals. Till and Palmer failed to isolate *A. lignieresi* from approximately 150 normal bovine tongues ; their failure suggests that colonisation of the lingual

mucosa does not take place, although it would be expected that in the process of rumination the ruminal organisms would be brought into intermittent contact with this surface.

The comparative ease with which *A. lignieresi* was recovered from ruminal contents suggests that it may be multiplying in this site. It is likely that the percentage of animals from which the organism was recovered is too low, since there is considerable immunological evidence that the majority of adult cattle have had some antigenic experience of *A. lignieresi* (Phillips, unpublished). It is unlikely, moreover, that the animals from which the positive cultures were obtained represent undetected cases of actinobacillosis since the incidence of this disease in cattle slaughtered at Edinburgh Abattoir, from which the material was drawn, is only 0·59-0·96 per cent. (Edinburgh Medical Officer of Health, 1955, 1956, 1957, 1958 and 1959).

SUMMARY

Organisms resembling *Actinobacillus lignieresi* in morphological and biochemical characters have been isolated from the ruminal contents of normal cattle. There is an antigenic relationship between these bacteria and pathogenic strains. The recovery of these organisms from normal cattle confirms the hitherto unsupported hypothesis of the commensal nature of *A. lignieresi*.

I wish to thank Mr J. Norval and his staff for facilities and help given in the collection of samples of ruminal contents and blood, Miss J. K. Buckley for technical assistance, Mr R. Ménard of Messrs Pfizer Ltd. for arranging the supply of oleandomycin and Dr A. Wilson Taylor for his interest in this work.

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SUMMARY

Organisms resembling *Actinobacillus lignieresi* in morphological and biochemical characters have been isolated from the ruminal contents of normal cattle. There is an antigenic relationship between these bacteria and pathogenic strains. The recovery of these organisms from normal cattle confirms the hitherto unsupported hypothesis of the commensal nature of *A. lignieresi*.

I wish to thank Mr J. Norval and his staff for facilities and help given in the collection of samples of ruminal contents and blood, Miss J. K. Buckley for technical assistance, Mr R. Ménard of Messrs Pfizer Ltd. for arranging the supply of oleandomycin and Dr A. Wilson Taylor for his interest in this work.

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# COMMENSAL ACTINOBACILLI FROM THE BOVINE TONGUE

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SEVERAL workers (Taylor, 1944; Till and Palmer, 1960) have suggested that *Actinobacillus lignieresi* may be present as a commensal in the mouth of susceptible animals, but hitherto the organism has not been isolated from this site. Organisms resembling *A. lignieresi* have been recovered from bovine ruminal contents (Phillips, 1961). This paper records the isolation from the tongues of normal cattle of 39 strains of organisms having morphological, cultural and biochemical characters similar to those of *A. lignieresi* isolated from pathological specimens.

## MATERIALS AND METHODS

*Media.* The media used in the identification of strains of actinobacilli were those described in a previous paper (Phillips, 1960). The medium used in the primary cultivation of material from tongues was the horse blood agar containing oleandomycin (1 $\mu$g. per ml.) and nystatin (200 units per ml.) used previously for the isolation of rumen actinobacilli (Phillips, 1961).

*Isolation of organisms from lingual swabs.* Mucus from the lateral aspect of the tongues of normal cattle was taken on swabs immediately after slaughter and inoculated on to antibiotic blood agar plates. After incubation, likely colonies were picked on to horse blood agar for further examination. The criteria for selection of colonies were (1) a diameter of 1–2 mm., (2) a smooth, moist appearance, and (3) a viscous character apparent when the colony was taken up on the inoculating loop.

## RESULTS

Swabs from the tongues of 105 cattle were examined. None of these animals showed any macroscopic evidence of actinobacillosis as judged by the usual meat inspection carried out in the abattoir. In some cases regurgitation of ruminal material at the time of slaughter had occurred, but such material was avoided in sampling.

The criteria used in the identification of actinobacilli were those already described (Phillips, 1960) and in particular the occurrence of the characteristic " Morse code " form was looked for in primary selection. Actinobacillus-like organisms were recovered from 39 (37 per cent.) of the swabs examined. All these organisms fermented glucose, fructose, xylose, sucrose, maltose, dextrin and mannitol without the production of gas, and failed to ferment trehalose, inulin, dulcitol and salicin within 14 days. All strains were able to grow on MacConkey's medium, to reduce nitrates to nitrites and to reduce methylene blue. None of the organisms examined gave a positive result with the methyl red test or with the tests for the production of indole, urease and ammonia, although hydrogen sulphide was formed by all. Differences between strains were observed with other fermentable substrates and biochemical tests, and these are shown in the table.

Several strains were agglutinated by antisera prepared against known pathogenic strains of *Actinobacillus lignieresi* and against strains of rumen actinobacilli.

## DISCUSSION

The slight differences in biochemical properties between the actinobacilli recovered from bovine tongues and classical strains of *Actinobacillus lignieresi*

isolated from lesions are similar to those recorded with rumen actinobacill (Phillips, 1961).

The comparative ease with which the organisms were isolated is surprising ir view of the failure of other workers to recover *A. lignieresi* from this site. Till anc Palmer failed to isolate *A. lignieresi* from 30 oral swabs taken from cattle anc cultured on nutrient agar and from 150 lingual swabs cultured on an antibiotic digest agar containing oleandomycin and neomycin. They isolated organisms tha had biochemical reactions similar to those of *A. lignieresi*, but were not agglutinatec by two specific antisera. The non-agglutination of these organisms by such a restricted number of specific antisera is not unexpected by comparison with the results obtained with rumen actinobacilli. Strains of *A. lignieresi* originating from

TABLE

*Biochemical reactions of actinobacilli from the tongue*

| Test | No. of strains | |
|---|---|---|
| | positive | negative |
| Fermentation of:— | | |
| Mannose | 33 (22*) | 6 |
| Galactose | 37 (23*) | 2 |
| Arabinose | 29 (3*) | 10 |
| Rhamnose | 10 (7*) | 29 |
| Lactose | 35* | 4 |
| Raffinose | 29* | 10 |
| Glycerol | 36 | 3 |
| Inositol | 16* | 23 |
| Catalase production | 36 | 3 |
| Voges-Proskauer test | 30 | 9† |
| Starch synthesis from :— | | |
| Glucose | 14 (12†) | 25 |
| Maltose | 21 (10†) | 18 |

* Late fermentation (negative at 24 hr; positive within 14 days).
† Weakly positive.

pathological lesions fall into at least six antigenic types (Phillips, unpublished) so that Till and Palmer using only two antisera may easily have overlooked many cultures that were in fact actinobacilli.

Previously the suggestion was made that Till and Palmer's results pointed to the fact that colonisation of the lingual epithelium by actinobacilli from the rumen did not take place. However, the higher percentage of actinobacilli recovered from bovine tongues compared with that found in the ruminal contents (10 per cent.) does not support this hypothesis, but would indeed point towards an active multiplication of the organisms in the mouth.

SUMMARY

Organisms resembling *Actinobacillus lignieresi* in morphological and biochemical characters have been isolated from tongues of normal cattle. These bacteria are similar to those recovered from bovine ruminal contents.

I wish to thank Mr J. Norval and his staff for facilities and help given in the collection of tongue swabs.

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# The Incidence of Agglutinating Antibodies to *Actinobacillus Lignieresi* in the Sera of Normal and Infected Cattle

BY

J. E. PHILLIPS

*Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, University of Edinburgh*

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J. E. PHILLIPS

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ACTINOBACILLOSIS of cattle is a disease affecting the alimentary tract, particularly the mouth and rumen and associated lymph-glands, and less frequently other tissues of the body, such as lung, skeletal muscle and liver. Differential diagnosis of this condition may be difficult and the use of a serological test as a diagnostic aid would be of value in some cases. The agglutination test is recommended by Wilson and Miles (1964) and Davies (1955), but the literature contains little experimental evidence of the value of this test. Thompson (1933) working in America had doubts about the value of the agglutination test as a completely satisfactory diagnostic method for cattle.

The present paper records the results obtained in the examination of sera from normal cattle and from animals affected with actinobacillosis made with a view to the development of a suitable serological test.

## MATERIALS AND METHODS

*Media.* Nutrient agar (infusion broth) used in the preparation of antigens was prepared as described by Cruickshank *et al.* (1960, pp. 190 and 195). Attempts were made to isolate *Actinobacillus lignieresi* from swabs taken from the tongues of cattle with clinical actinobacillosis; cultures were grown on the horse blood agar containing oleandomycin (1 $\mu$g. per ml.) and nystatin (200 units per ml.) used previously for the isolation of actinobacilli from the rumen and tongues of normal cattle (Phillips, 1961, 1964).

*Isolation of organisms from lesions.* The methods used for the isolation and identification of *A. lignieresi* from necropsy material were as described by Phillips (1960).

*Preparation of antigens.* Bacterial suspensions for use in agglutination tests were obtained by growing the organisms on the surface of nutrient agar slopes in 120-ml. medical flat bottles inoculated with 5 ml. of an overnight broth culture. After 18–20 hr at 37° C the growth was washed off with the broth inoculum and steamed at 100° C for 2 hr. The bacterial cells were separated by centrifugation and suspended in saline (0·85 per cent. NaCl) solution to a density corresponding to a scale reading of 1·5 on an EEL portable colorimeter (Evans Electroselenium Ltd) with a green (OGR1) filter. The density was approximately that of Brown's opacity standard no. 1. This is referred to as " heated antigen ".

A second type of suspension was also employed in which heating for 2 hr was omitted and the cells were suspended in saline containing 0·2 per cent. formaldehyde (formolised antigen).

*Collection of sera.* Blood from adult cattle slaughtered at the local abattoir was collected either from the axillary vessels or from the chambers of the heart when

this organ was opened for inspection. Tissues were taken for cultural examination from any animal showing lesions with the macroscopic appearance of actinobacillosis. Two hundred and eight sera submitted to the Department for examination for contagious bovine abortion were also examined. Twenty-one young cattle in the herd attached to the Veterinary School were bled from the jugular vein at monthly intervals over a period of 20 mth. One hundred and seventy-three samples of blood from cattle clinically affected with actinobacillosis were submitted by practising veterinary surgeons.

*Stock cultures.* The occurrence of several antigenic types of *A. lignieresi* has been noted previously (Phillips, 1964) and five strains of the organism, chosen to cover the types detected up to the present time in pathological material examined in this laboratory, were used in the preparation of antigens. The origins of these strains were as follows: strain A2, bovine tongue; strain A3, ovine lung; strain A7, ovine abscess; strains A13 and A15, bovine retropharyngeal lymph-gland. These strains were maintained in cooked-meat medium and were also preserved by drying *in vacuo*.

*Agglutination tests.* Each serum was set up in serial doubling dilutions against a number of antigens. The tests were done in 2 in. $\times \frac{1}{4}$ in. (50 mm. $\times$ 6 mm.) test-tubes (Durham tubes) and incubated 18–20 hr in a waterbath at 56° C. The first serum dilution was 1 in 10 in every case, but the range varied with the purpose of the test. Sera from normal animals were examined initially in the range 1 in 10 to 1 in 160 and any sera showing agglutination at 1 in 160 were further examined in a dilution series from 1 in 10 to 1 in 20,480. In addition, a number of sera with titres less than 1 in 160 were examined in this range of dilutions to test for the presence of a prozone.

Sera from clinical cases of actinobacillosis and from animals showing lesions at meat inspection were examined over the range 1 in 10 to 1 in 20,480.

## Results

### *Normal adult cattle*

Five hundred and fifty-two slaughterhouse samples of blood from normal animals were collected and subjected to agglutination tests with heated antigens. The results are given in table I. Prozones were found in only 4 tests and 3 of these were with one serum. The highest titre occurring with a prozone was 1 in 320; this was obtained with antigen A2. In no case was the prozone extensive and, in two cases, the titre was only 1 in 80.

The results obtained with 208 blood samples submitted to the laboratory for examination for contagious bovine abortion are given in table II. Prozones were present on only two occasions and both of these were obtained with the same serum, the titre with each antigen being 1 in 160. One serum brought about agglutination at a dilution higher than 1 in 320, the titre being 1 in 1280. The animals from which these laboratory samples were derived have been assumed not to be clinical cases of actinobacillosis, but no post-mortem examinations were available to confirm this.

With both slaughterhouse and laboratory samples, the majority of sera gave agglutination at dilutions no higher than 1 in 20, with the exception that antigen A3, which appeared to be especially sensitive, was agglutinated by most of the sera in dilutions of from 1 in 40 to

1 in 160. In no case, however, did agglutination occur at a higher dilution than 1 in 160 with antigen A3. In two tests, each with antigens A2 and A15, agglutination occurred at a dilution of 1 in 320. Very few sera in these two groups of samples were without any activity

TABLE I

*Agglutination titres of 552 samples of normal bovine serum taken at slaughter in tests against heated suspensions of five strains of Actinobacillus lignieresi*

| Strain tested as agglutinable suspension | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|
| | | <10 | 10–20 | 40–160 | 320 | >320 |
| A2 | 1 | 279 | 231 | 40 (4) | 2 | 0 |
| A3 | 0 | 14 | 99 | 439 (74) | 0 | 0 |
| A7 | 0 | 71 | 387 | 94 (2) | 0 | 0 |
| A13 | 1 | 191 | 317 | 44 (0) | 0 | 0 |
| A15 | 2 | 310 | 217 | 24 (1) | 1 | 0 |

* Titres are reciprocals of the highest dilution of serum giving agglutination of the test strain of actinobacillus. Figures in brackets are numbers of sera giving complete agglutination at a dilution of 1 in 160.

whatsoever on the antigens employed; only 4 laboratory samples from calves and 8 slaughterhouse samples gave completely negative results.

The use of formolised antigens to examine sera from clinical cases was introduced some time after the investigation was begun and therefore not all sera from normal animals were tested with these antigens.

TABLE II

*Agglutination titres of 208 samples of bovine serum collected for examination for contagious bovine abortion in tests against heated suspensions of five strains of A. lignieresi*

| Strain tested as agglutinable suspension | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|
| | | <10 | 10–20 | 40–160 | 320 | >320 |
| A2 | 0 | 47 | 110 | 51 (0) | 0 | 0 |
| A3 | 1 | 2 | 20 | 186 (26) | 0 | 0 |
| A7 | 1 | 13 | 91 | 104 (5) | 0 | 0 |
| A13 | 0 | 37 | 81 | 90 (2) | 0 | 0 |
| A15 | 0 | 41 | 81 | 84 (2) | 1 | 1 |

* See footnote to table I.

However, a comparison of the two types of antigen was made with 92 slaughterhouse samples selected at random and the results of tests with 4 antigenic strains are shown in table III. In general the reactions with the two types of antigen were similar, but the formolised antigens were slightly more sensitive than the heated ones. In no case was

there more than one dilution tube difference in the results with the two types of antigen.

*Normal young cattle*

The absence of antibodies to *A. lignieresi* in the sera of calves noted in the first part of the work prompted a more extensive examination of samples from young stock. Blood samples were drawn at monthly intervals from all calves in the dairy herd attached to the Veterinary School and were examined against heated antigens. The results are shown for the various age groups in table IV. Included in this table is a score based upon the agglutination results with all five antigens (see

TABLE III

*Agglutination titres of 92 sera collected from normal cattle at slaughter in tests against four heated and four formolised suspensions of A. lignieresi*

| Agglutinable suspension | | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|
| Preparation | Strain | | <10 | 10–20 | 40–160 | 320 | >320 |
| Heated . . | A2 | 0 | 35 | 51 | 6 (0) | 0 | 0 |
| | A3 | 1 | 1 | 12 | 79 (1) | 0 | 0 |
| | A7 | 0 | 9 | 69 | 14 (0) | 0 | 0 |
| | A13 | 0 | 23 | 62 | 7 (0) | 0 | 0 |
| Formolised . | A2 | 0 | 21 | 63 | 8 (0) | 0 | 0 |
| | A3 | 0 | 0 | 15 | 76 (1) | 1 | 0 |
| | A7 | 0 | 6 | 75 | 11 (1) | 0 | 0 |
| | A13 | 0 | 26 | 61 | 5 (0) | 0 | 0 |

* See footnote to table I.

footnote to table). No serum agglutinated any antigen at a dilution higher than 1 in 160 and no prozone was observed.

Antibodies to *A. lignieresi* were absent from the sera of very young calves (1–2 wk) but with increasing age in the animals a gradual increase of such antibodies was noticed, the titres reaching their maxima (*c.* 20 marks) at about 1 yr of age. The table shows some fluctuation in the scores in older animals, but the number of animals involved is small in the older age groups.

For comparison a similar score was calculated for normal adult cattle from data obtained from the slaughterhouse samples and the average score was found to be 14·6.

*Clinical and slaughterhouse cases of actinobacillosis*

During the course of collecting slaughterhouse blood samples, 21 cases of actinobacillosis were seen. *A. lignieresi* was isolated from the tongue in 10 cases, from lymph-glands in the head in 5, from cheek lesions in 3, from the palate in 2, and from the reticulum in 1. The serum from each animal was tested against the five heated antigens

| Age of animals (wk) | No. of sera showing stated titre in test with | | | | | | | | | | | | | | | No. of animals examined | Average score for animals examined (marks)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | strain A2 | | | strain A3 | | | strain A7 | | | strain A13 | | | strain A15 | | | | |
| | <10 | 10–20 | 40–160 | <10 | 10–20 | 40–160 | <10 | 10–20 | 40–160 | <10 | 10–20 | 40–160 | <10 | 10–20 | 40–160 | | |
| 0–4 | 10 | 2 | 0 | 12 | 0 | 0 | 12 | 0 | 0 | 10 | 1 | 1 | 11 | 1 | 0 | 12 | 1·0 |
| 5–8 | 9 | 10 | 0 | 17 | 2 | 0 | 17 | 2 | 0 | 17 | 2 | 0 | 14 | 5 | 0 | 19 | 1·6 |
| 9–12 | 8 | 11 | 0 | 11 | 6 | 2 | 19 | 0 | 0 | 14 | 5 | 0 | 14 | 5 | 0 | 19 | 2·9 |
| 13–16 | 11 | 6 | 0 | 1 | 13 | 3 | 11 | 6 | 0 | 11 | 6 | 0 | 13 | 4 | 0 | 17 | 5·2 |
| 17–20 | 9 | 8 | 1 | 0 | 10 | 8 | 9 | 8 | 1 | 13 | 5 | 0 | 11 | 7 | 0 | 18 | 7·9 |
| 21–24 | 4 | 9 | 0 | 1 | 6 | 6 | 4 | 9 | 0 | 5 | 7 | 1 | 9 | 4 | 0 | 13 | 10·4 |
| 25–28 | 7 | 13 | 0 | 1 | 8 | 11 | 7 | 13 | 0 | 8 | 10 | 2 | 9 | 9 | 2 | 20 | 10·2 |
| 29–32 | 5 | 11 | 0 | 0 | 4 | 12 | 1 | 12 | 3 | 5 | 9 | 2 | 10 | 5 | 1 | 16 | 14·3 |
| 33–36 | 6 | 9 | 1 | 0 | 4 | 12 | 0 | 14 | 2 | 3 | 11 | 2 | 5 | 11 | 0 | 16 | 14·5 |
| 37–40 | 4 | 9 | 0 | 0 | 3 | 10 | 1 | 11 | 1 | 4 | 8 | 1 | 7 | 6 | 0 | 13 | 12·3 |
| 41–44 | 5 | 9 | 1 | 0 | 2 | 13 | 2 | 9 | 4 | 1 | 10 | 4 | 6 | 7 | 2 | 15 | 16·7 |
| 45–48 | 4 | 7 | 1 | 0 | 4 | 8 | 0 | 11 | 1 | 2 | 9 | 1 | 5 | 7 | 0 | 12 | 13·8 |
| 49–52 | 1 | 7 | 1 | 0 | 2 | 7 | 0 | 8 | 1 | 0 | 9 | 0 | 2 | 7 | 0 | 9 | 16·8 |
| 53–56 | 2 | 10 | 0 | 0 | 1 | 11 | 0 | 9 | 3 | 1 | 9 | 2 | 2 | 8 | 2 | 12 | 19·8 |
| 57–60 | 1 | 12 | 0 | 0 | 0 | 13 | 0 | 9 | 4 | 0 | 9 | 4 | 2 | 6 | 5 | 13 | 22·0 |
| 61–64 | 2 | 8 | 1 | 0 | 0 | 11 | 1 | 9 | 1 | 2 | 7 | 2 | 0 | 8 | 3 | 11 | 19·5 |
| 65–68 | 1 | 10 | 1 | 0 | 1 | 11 | 0 | 8 | 4 | 2 | 8 | 2 | 2 | 9 | 1 | 12 | 20·1 |
| 69–72 | 2 | 7 | 0 | 0 | 2 | 7 | 0 | 6 | 3 | 2 | 5 | 2 | 0 | 6 | 3 | 9 | 19·2 |
| 73–76 | 1 | 3 | 1 | 0 | 0 | 5 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 3 | 2 | 5 | 21·2 |
| 77–80 | 3 | 6 | 2 | 0 | 1 | 10 | 0 | 6 | 5 | 4 | 4 | 3 | 0 | 5 | 6 | 11 | 22·2 |
| 81–84 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 3 | 0 | 0 | 1 | 2 | 3 | 24·0 |
| 85–88 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 22·5 |
| 89–92 | 2 | 2 | 1 | 0 | 1 | 4 | 0 | 2 | 3 | 4 | 0 | 1 | 0 | 4 | 1 | 5 | 19·6 |
| 93–96 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 10·0 |
| 97–100 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 20·0 |

* System of scoring: agglutination partial at serum dilution 1 in 10, 1 mark; complete at 1 in 10, 2 marks; partial at 1 in 20, 3 marks; complete at 1 in 20, 4 marks; partial at 1 in 40, 5 marks; complete at 1 in 40, 6 marks; partial at 1 in 80, 7 marks; complete at 1 in 80, 8 marks; partial at 1 in 160, 9 marks; complete at 1 in 160, 10 marks; and so on.

and, in addition, against heated and formolised antigens prepared with the strain of *A. lignieresi* isolated from the animal (homologous organism). The results are given in table V. A prozone was present with one or more antigens with 12 of the sera and in most cases the titre of the serum was greater than 1 in 160. The highest titre was 1 in 2560.

Of the 7 sera giving a prozone with antigen A2, one had a titre less than 1 in 160 and two had a titre of 1 in 160. With antigen A13 one of the 7 sera showing a prozone had a titre less than 1 in 160. In all other cases the sera showing prozones had titres greater than 1 in 160.

TABLE V

*Agglutination titres of sera from 21 bovine carcasses with actinobacillosis observed at meat inspection in tests against heated and formolised suspensions of A. lignieresi*

| Agglutinable suspension | | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|
| Preparation | Strain | | <10 | 10–20 | 40–160 | 320 | >320 |
| Heated . . | A2 | 7 | 7 | 3 | 6 (2) | 1 | 4 |
| | A3 | 1 | 0 | 0 | 20 (3) | 1 | 0 |
| | A7 | 0 | 4 | 9 | 8 (0) | 0 | 0 |
| | A13 | 7 | 4 | 7 | 4 (1) | 1 | 5 |
| | A15 | 7 | 6 | 5 | 3 (1) | 2 | 5 |
| Heated . . | Homologous strain | 5 | 4 | 2 | 4 (2) | 1 | 10 |
| Formolised . | Homologous strain | 2 | 5 | 2 | 6 (2) | 0 | 8 |

* See footnote to table I.

Seven of these sera from animals known to be infected with *A. lignieresi* gave completely negative results; their titres fell within the values obtained with normal cattle even in tests with antigens prepared from the infecting organism. When the critical titre was taken as 1 in 640, the agglutination tests with the five standard antigens detected only 6 (28·6 per cent.) of the infected animals. If tests with the antigens prepared from the infecting organism were also considered the number of infected animals detected increased to 12 (57·2 per cent.). When the critical titre was taken as only 1 in 320 the number of cases detected in tests with the five standard antigens rose to 9 (42·9 per cent.) and the number detected in tests with the homologous antigens rose to 14 (66·7 per cent.).

With the cooperation of several practising veterinary surgeons blood samples and lingual swabs were obtained from 173 cases of clinical actinobacillosis of the tongue. In every case the swabs failed to yield organisms identifiable as *A. lignieresi.* To assess the survival rate of the organism on swabs, a number of " positive " swabs were prepared and subjected to similar conditions of storage to those from

clinical cases of actinobacillosis, viz. standing at room temperatures and transmission through the post. Two groups of " positive " swabs were used, one prepared by dipping sterile swabs in nutrient broth cultures of four strains of *A. lignieresi* and the other by taking oral mucus from a bovine animal on to the swabs and then dipping the swabs into nutrient broth cultures. Immediately the swabs were prepared they were inoculated on antibiotic blood agar and this was repeated 3 days later after they had been sent through the post or had lain at room temperature. On the plates prepared immediately, colonies of *A. lignieresi* were easily recognisable and were isolated for

TABLE VI

*Agglutination titres of sera from 173 cattle with clinical actinobacillosis in tests against heated and formolised suspensions of five strains of A. lignieresi*

| Agglutinable suspension | | Total no. of sera examined | No. of sera showing prozone | No. of sera showing titre* of | | | | |
|---|---|---|---|---|---|---|---|---|
| Preparation | Strain | | | <10 | 10–20 | 40–160 | 320 | >320 |
| Heated | A2 | 173 | 41 | 31 | 42 | 25 (1) | 13 | 62 |
| | A3 | 173 | 3 | 39 | 38 | 73 (9) | 12 | 11 |
| | A7 | 173 | 2 | 20 | 44 | 102 (11) | 4 | 3 |
| | A13 | 170 | 0 | 21 | 73 | 72 (2) | 2 | 2 |
| | A15 | 156 | 0 | 34 | 30 | 87 (4) | 3 | 2 |
| Formolised | A2 | 156 | 59 | 19 | 41 | 22 (0) | 7 | 69 |
| | A3 | 144 | 1 | 57 | 49 | 30 (3) | 5 | 3 |
| | A7 | 137 | 3 | 3 | 20 | 96 (16) | 9 | 9 |
| | A13 | 144 | 4 | 9 | 25 | 53 (5) | 20 | 37 |
| | A15 | 16 | 0 | 2 | 6 | 8 (0) | 0 | 0 |

* See footnote to table I.

identification. On the plates prepared from the swabs after transmission through the post *A. lignieresi* was not detected; the swabs of nutrient broth cultures were now sterile, and those with oral mucus showed numerous contaminating organisms but no actinobacilli.

The serum samples were examined in agglutination tests with both heated and formolised antigens and the results are given in table VI. Sixty-nine sera produced prozones with one or more of the antigens and most of these sera had titres greater than 1 in 160. The highest titre, found in two sera, was 1 in 20,480. The distribution of the titres of the sera showing prozones is given in table VII.

Table VI shows that a considerable number of sera from cases of actinobacillosis produced agglutination at titres higher than were obtained with normal sera. Not all sera were tested with both heated and formolised antigens, but of the 156 that were examined in this way, 68 (43·6 per cent.) gave completely negative results, their titres falling within the values obtained with normal cattle (critical titre 1 in 640). Sixty-one of the remaining sera (39·1 per cent.) agglutinated one or more of the heated and formolised antigens at or above this

critical titre; 8 (5·1 per cent.) gave a positive result only with the heated antigens and 19 (12·2 per cent.) were positive only with the formolised one. When the critical titre was taken as 1 in 320, 99 (63·5 per cent.) of the sera gave positive results and 57 (36·54 per cent.) gave titres that were within the normal range for cattle. Of the positive sera at this critical level, 75 (48·1 per cent.) agglutinated one or more of both types of antigen to a high titre; 11 (7·1 per cent.) gave a high titre only with the heated antigen and 13 (8·33 per cent.) did so only with the formolised one.

TABLE VII

*Agglutination titres in sera from clinical cases of actinobacillosis showing prozones*

| Agglutinable suspension | | No. of sera showing titre of | | | No. of sera examined |
|---|---|---|---|---|---|
| Preparation | Strain | <160 | 160 | >160 | |
| Heated . . | A2 | 0 | 0 | 41 | 41 |
| | A3 | 2 | 0 | 1 | 3 |
| | A7 | 0 | 1 | 1 | 2 |
| Formolised . . | A2 | 0 | 1 | 58 | 59 |
| | A3 | 0 | 0 | 1 | 1 |
| | A7 | 0 | 0 | 3 | 3 |
| | A13 | 0 | 0 | 4 | 4 |

## DISCUSSION

Few records are available of the occurrence of antibodies to *Actinobacillus lignieresi* in the serum of normal bovine animals. Thompson (1933) examined the serum from one normal animal with suspensions of six strains of *A. lignieresi* and obtained titres of 1 in 20 or 1 in 40. Davies (1955) stated that serum from healthy animals never agglutinates at a dilution of 1 in 20. The present results suggest that these values are low and that titres up to 1 in 160 may be considered normal. Such titres are particularly likely to be obtained when certain strains of *A. lignieresi* (e.g. A3) are used as antigen. Although none of the slaughterhouse samples was taken from an animal showing gross lesions of actinobacillosis, minor lesions may been have overlooked during the course of meat inspection and this might account for the three " healthy " animals showing a titre of 1 in 320.

The occurrence of antibodies to *A. lignieresi* in the sera of cattle is to be expected in view of the occurrence of actinobacilli in the rumens and on the tongues of normal adult cattle (Phillips, 1961, 1964). It has been suggested (Phillips, 1961) that *A. lignieresi* may play some part in microbial digestion in the rumen, perhaps through its ability to synthesise starch (Phillips, 1960). In these circumstances it is likely that some time will elapse before a population of commensal actinobacilli establishes itself in the rumen of a young animal and before any

antigenic stimulus can reach the antibody-forming tissues. The gradual appearance of antibodies to *A. lignieresi* in the sera of young cattle is in agreement with this hypothesis.

A striking feature of the results obtained with the sera from clinical and slaughterhouse cases of actinobacillosis was the occurrence of prozones in tests with many of the sera, including some whose agglutinin titre was rather low. This suggests that the development of a prozone in a diagnostic test would be strongly indicative of the presence of infection.

The failure of the agglutination test to detect 43·6 per cent. of the clinical cases of actinobacillosis might be due to the presence in the sample of wrongly diagnosed cases or of cases caused by antigenic types of *A. lignieresi* other than those employed as antigens. The geographical distribution of the places of origin of the infected animals is not known precisely, since many of the animals were examined in the local abattoir, which handles cattle from Scotland and Ireland. It is possible that different antigenic types of actinobacillus occur in other parts of this country and elsewhere in the world.

The short survival time of *A. lignieresi* on artificially infected mouth swabs supports the findings of Till and Palmer (1960) who showed that the organism was not recoverable later than 5 days after artificial infection of hay and straw. Stock laboratory cultures of *A. lignieresi* tend to die rapidly unless preserved in the dried state. Blood agar cultures of the organism will not remain viable beyond a week, and even on Dorset egg or in cooked-meat medium subcultures have to be made every 14–21 days.

SUMMARY

Antibodies to *Actinobacillus lignieresi* were demonstrated in sera from normal adult cattle in titres up to 1 in 160. Very young calves did not possess such antibodies but gradually acquired them during their first year of life.

Most sera from cattle clinically affected with actinobacillosis and from slaughterhouse cases of the disease showed higher levels of antibody than normal animals and the occurrence of a prozone in tests with such samples was a notable feature.

I am indebted to Mr J. Norval and his staff for facilities and help given in the collection of blood samples and to the many veterinary surgeons who supplied me with samples from clinical cases of actinobacillosis. My thanks are due also to Miss I. Pow for technical assistance and to Mr D. Laing and his staff for assistance in the work on young cattle.

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# The Antigenic Structure and Serological Typing of *Actinobacillus lignieresi*

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In their first description of Actinobacillus lignieresi Lignières and Spitz (1902) mentioned the use of a serum agglutination test as a diagnostic measure for actinobacillosis of cattle, but, whilst remarking upon a variability in the speed of the reaction with sera from a number of experimental animals, did not investigate the possible existence of different antigenic types of the organism.

This possibility has since been considered by a number of workers, but the absence of conclusive results prompted Wilson and Miles (1955, p. 472), in their description of the antigenic structure of A. lignieresi, to the comment "little known". Agglutination of A. lignieresi by antisera has been investigated with variable results, sometimes all strains being found to be identical (Davies and Torrance, 1930) and sometimes antigenic differences being noted (Thompson, 1933). Tunnicliff (1941) drew attention to the difficulty of identifying A. lignieresi by serological means. Antigenic interrelationships between strains of A. lignieresi of human and those of bovine origin have been observed in complement-fixation studies (Beaver and Thompson, 1933) and agar-gel precipitation studies (Pathak and Ristic, 1962), and antigens have been found in A. lignieresi that are also present in other bacteria, viz. Pfeifferella mallei and Pf. whitmori (Beaver and Thompson) and Actinobacillus equuli (Bacterium viscosum equi) (Vallée, Thibault and Second, 1963).

All previous workers have examined only small numbers of strains and it is the purpose of this paper to describe the antigenic characters of 218 strains of A. lignieresi isolated from lesions of

actinobacillosis in cattle and sheep.

## MATERIALS AND METHODS

Source of strains of Actinobacillus lignieresi. The strains were isolated from lesions of actinobacillosis in cattle and sheep. The mode of isolation, as well as the morphological, cultural and biochemical characters of the organisms, have been described previously (Phillips, 1960). Five strains obtained from the National Collection of Type Cultures (no. 4189, 4191, 4975, 4976 and 4985) were included in the investigation.

Media. Nutrient agar containing meat infusion broth was prepared as described by Cruickshank et al. (1960, pp. 190 and 195) and used for growing cultures for the preparation of the antigens.

### Preparation of antigens

Bacterial suspensions that were to be used as antigens in the tube agglutination tests were obtained by growing the organisms on the surface of nutrient agar slopes in 120-ml. flat bottles inoculated with 5 ml. of an overnight broth culture. After 18-20 hr at 37°C the growth was washed off with the broth inoculum and steamed at 100°C for 2 hr. The bacterial cells were separated from the broth by centrifugation and were resuspended in saline (0.85 per cent. NaCl) solution to a density corresponding to a scale reading of 1.5 on an EEL portable colorimeter (Evans Electroselenium Ltd.) with a green (OGR1) filter. This density was approximately that of Brown's opacity standard no. 1 (see Cruickshank et al., p. 305). Suspensions

of all strains of organisms subjected to the tube agglutination test were prepared in this way and are referred to as "heated antigens".

Two other types of suspension were used with a small number of strains in both of which the heating for 2 hr was omitted and the cells after centrifugal separation were resuspended in saline (living antigen) or in saline containing 0.2 per cent. formaldehyde (formolised antigen). The living antigen was used at once but heated and formolised antigens were stored in the refrigerator and used over periods of several weeks.

Heavy suspensions of all strains of A. lignieresi for use in the slide agglutination test were obtained by growing the organisms on nutrient agar slopes in 120-ml. flat bottles, washing off the growth from one slope with 1 ml. saline and steaming the suspension for 2 hr. This suspension is referred to as "heated slide antigen". In some experiments the steaming was omitted (living slide antigen).

Bacterial suspensions used for the immunisation of animals were prepared in the same way as the "heated antigens" for the tube agglutination test except that the final density was adjusted to Brown's opacity standard no. 8 (heat-killed vaccine). A formolised vaccine was also employed and consisted of a nutrient broth culture of the organism incubated for 20 hr at 37°C and killed by the addition of 0.04 per cent. formaldehyde. Formolised vaccine was followed by 1 or 2 injections of a living nutrient broth culture.

## Preparation of antisera

Rabbits were used for the preparation of antisera against 22 strains of *A. lignieresi*. Six antisera were obtained from goats. Prior to inoculation, rabbits and goats were bled to test for natural antibodies against the strains of organisms to be used for immunisation. Rabbits received 7 intravenous injections at intervals of 4 days, the first and second doses being 0.25 and 0.5 ml., respectively, and later doses were 1 ml. Injections were made into the marginal vein of the right ear, the left ear being reserved for bleeding by venepuncture. Test bleedings were made 7-10 days after the last injection and if the agglutinating titre was satisfactory further bleedings were made from the ear vein or by heart puncture. Antisera were preserved with 0.5 per cent. phenol by adding 1 ml. of saline containing 5 per cent. phenol to each 10 ml. of serum.

Antisera used for slide agglutination tests were diluted with saline containing 0.5 per cent. phenol until they gave readily observable agglutination of the homologous antigen within 1 min. on a slide.

## Agglutination tests

Slide agglutination tests were carried out on a glass plate mounted over a black background with oblique lighting so that clumping of the bacterial cells could be seen without difficulty. Slide antigen was deposited on a glass plate with a large inoculating loop, an equal volume of antiserum was added and the whole was mixed

with the same loop.

Tube agglutination tests were done in 2 in. x ¼ in. (50 mm. x 6 mm.) tubes and incubated 18–20 hr in a waterbath at 56°C. Serial doubling dilutions of serum were made from 1 in 10 upwards, the range covered being determined by the titre of the antiserum for its homologous antigen. Titres are expressed as the highest dilution of serum, in final mixture with the bacteria, that gave agglutination visible to the naked eye.

Absorption of antisera. Absorbing stains of A. lignieresi were grown overnight on nutrient agar slopes in the same way as for preparation of antigens. The growth from two slopes was washed off with 2-3 ml. sterile saline. In some cases the suspension was then steamed for 2 hr or else the living organisms were used. The bacterial cells, living or heated, were separated by centrifugation and resuspended in 2 ml. of a 1 in 5 dilution of the serum to be absorbed. After incubation at 37°C for 2 hr the serum-antigen mixture was allowed to stand overnight in the refrigerator and the cells were then removed by centrifugation. A single-tube agglutination test with equal volumes of the absorbed serum and the tube antigen of the absorbing strain of A. lignieresi was used to show that absorption was complete. Finally, a tube agglutination test was set up with the absorbed serum against its homologous antigen, the serial dilutions being taken two steps beyond the known titre of the unabsorbed serum.

Examination for capsulation. Cultures grown on 0.1 per cent. dextrose agar were examined for the presence of capsules and extracellular slime

by the wet-film India ink method of Duguid (1951).

Demonstration of extracellular antigenic material. Cultures of A. lignieresi grown on 0.1 per cent. dextrose agar for 18-20 hr were washed off with saline and the suspensions were incubated at 37°C for 2 hr. The cells were deposited by centrifugation and the supernatant fluid was examined in precipitation tests against A. lignieresi antisera prepared with living antigens. The precipitation tests were carried out in capillary tubes that were partially filled with serum followed by the saline extract; the tubes were supported in "Plasticine" during incubation at 37°C for 2 hr.

## RESULTS

### Slide agglutination tests

Heated antigens of each of the 28 strains of Actinobacillus lignieresi used to prepare antisera were tested against each of the antisera and the results are shown in table I. The organisms examined could be divided into six types on the basis of these results, all except two of the strains (A49 and A20) being typed in this way. There appeared to be a degree of antigenic overlap between some of the types, cross reactions with organisms in heterologous types being shown most markedly with the three antisera against strains A13, A47 and A49. Strain A47, whilst giving an agglutination pattern similar to organisms of type 1, produced an antiserum which agglutinated organisms of types 2 and 4 in addition to those of type 1. In the same way strain A13, which fell into the type 5 agglutination pattern,

gave an antiserum reacting with organisms of types 2, 4 and 6. Although strain A49 was one of the two strains that it was not possible to type, its antiserum agglutinated organisms in types 2, 4, 5 and 6.

Slide agglutination tests of the heated antigens of all 218 strains of A. lignieresi were made against the 28 antisera and the distribution of these strains between the six antigenic types is given in table II. Only 15 strains could not be placed into the types previously demonstrated and of these, four underwent spontaneous agglutination in saline. The other 11 strains, although agglutinating with several of the antisera did not correspond with the agglutination patterns of any of the types, but an interesting feature was the fact that all these strains agglutinated with antiserum A4, as did strains A49 and A20 (see table I). Twenty-five organisms differed slightly from the strains that were placed in type 1 in not agglutinating with one or two of the 10 sera characterising the type and these have been designated as belonging to subtype 1a strains. Similarly, three strains that differed slightly from the agglutination pattern characterising type 4 organisms have been designated as belonging to subtype 4a.

Slide agglutination tests on the five strains from the National Collection of Type Cultures (NCTC) showed that they also fitted into the agglutination patterns shown by other strains. Strains NCTC 4975 and 4976 were of antigenic type 1 and the 3 other strains were of antigenic subtype 4a.

## Tube agglutination tests

Twenty-four antisera against the organisms that could by typed were tested against heated antigens of the other strains of the same antigenic type to ascertain the agglutinating titres.

The results of these tests with organisms of type 1 are shown in table III from which it can be seen that all antisera to organisms of this type agglutinated all strains within the type, even antisera A100 and A133 which had not agglutinated all the strains in the slide tests. Antisera to organisms of types 2 (A3, A5), 4 (A7, A33, A53), 5 (A13, A43, A75) and 6 (A15, A17), as well as one antiserum to a type - 3 organism (A6), agglutinated all organisms in the homologous type to the full titre of the antiserum. Antiserum A14 (type 3) was exceptional; it agglutinated A6 bacilli only to a low titre.

Antisera A13 and A47, which in the slide agglutination tests were found to give a more extensive pattern of cross agglutination than other antisera, were examined in tube agglutination tests against the heated antigens of the strains that they agglutinated in the slide tests. The results are shown in table IV, from which it can be seen that, with most antigens, agglutination occurred only in low dilutions of antiserum.

## Absorption tests

Each of the 24 sera against the organisms that could be typed were absorbed with the organisms of the same antigenic type as their homologous organism and then tested against the absorbing and homologous strains. Table III shows the results of absorbing the antisera to 12 type-1 strains

with each of the eleven heterologous type-1 strains. In 122 of the 132 absorptions there was complete absorption of antibodies and in the other 10 absorptions the antibodies were reduced to a low level. These results suggest that there was complete antigenic homogeneity between the 12 organisms tested. Two antisera to organisms of each of types 2 (A3, A5) and 3 (A6, A14) and three antisera to organisms of each of types 4 (A7, A33, A53) and 5 (A13, A43, A75) were absorbed with the heterologous strains of the same antigenic type, and this resulted in the complete removal of agglutinins for the homologous strain in all the sera except one, in which antibodies had been reduced to a low level. The only exceptional finding was that the two representative strains of type 3, A6 and A14, failed to absorb antibodies from the heterologous antiserum, and appeared to differ from each other.

Antisera A13, A47 and A49 were absorbed with the strains of A. lignieresi with which they had cross-agglutinated in slide tests. The results with antisera A13 and A47 are shown in table IV. Antiserum A13 was absorbed with eight strains and in every case, although antibodies against the absorbing strain were removed, the titre of the serum for the homologous organism remained at the same level as that of the unabsorbed serum. Three of the absorbed A13 antisera were further examined by slide agglutination against the strains used for absorption. For comparison the three strains of type 5 were included as also was antiserum A13 (type 5) absorbed with strain A75 (type 5).

The results of these tests are shown in table V, from which it is clear that although the three absorbing strains A5, A7 and A17 belong to different antigenic types (2, 4 and 6) they removed the antibodies not only for organisms of their own type but also for those of the other two types and for strains A49 and A20 which were not typed. The antisera obtained by these absorptions were specific for organisms of antigenic type 5.

Antiserum A47 (type 1) was absorbed with five cross-agglutinating strains and in each case there was complete absorption of antibodies for the absorbing strain, but the titre for heated antigen A47 was unchanged. Antiserum A49 did not produce visible agglutination of the homologous heated antigen in tube agglutination tests, but the serum would agglutinate slide antigens. Absorption of this serum without prior dilution was performed with strains A3, A5, A7, A13, A17, A33, A43, A53 and A75, and the absorbed serum was then tested by the slide method for the presence of antibodies to the absorbing and homologous strains. The results with antiserum A49 differed from those with the antisera A13 and A47, since each absorbing strain produced complete removal of antibodies for the homologous strain.

The antigenic relationships of the eleven strains of A. lignieresi that could not be typed but were agglutinated by antiserum A4 (table II) was investigated by absorbing antiserum A4 with strain A49 and then testing it against slide antigens of the eleven strains. This absorption resulted in the removal of antibodies for all the untyped strains, but

the serum still agglutinated heated antigen A4 to full titre and still gave agglutination of slide antigens of a number of strains of antigenic type 1.

Agglutination of living and formolised antigens

The characteristic viscid colonies observed in freshly isolated cultures of A. lignieresi suggested that some kind of capsular material might be present and the existence of antigens associated with living but not with killed bacterial cells was investigated. Slide agglutination tests were carried out on living and heated suspensions of strains A1, A3 and A7 using two groups of antisera; antisera A1, A2, A3 and A33 had been prepared against heated bacterial cells and antisera A5, A6, A7 and A15 against living bacterial cells. The results obtained with these tests are shown in table VI. With each bacterial strain tested, agglutination of the living organisms was obtained with all three sera that had been prepared against living organisms (A5, A6, A7) although the heated organisms gave patterns of agglutination that were quite distinct and corresponded to those of the three antigenic types represented by the strains tested, viz. types 1, 2 and 4.

Tube agglutination tests were used to test heated, formolised and living antigens of three A. lignieresi strains against three pairs of antisera prepared with living and heated vaccines of the same three strains. The results are shown in table VII. Strains A46 and A47 are both of antigenic type 1 but strain A49 is an organism that was not typed (table II). Antiserum A49 (living), whilst not agglutinating

any of the heated antigens, did agglutinate formolised and living antigens of all three strains. Likewise, antisera A46 (living) and A47 (living) agglutinated formolised and living antigens A49 but not heated antigen of the same organism. Antisera A46 (heated) and A47 (heated) agglutinated the heated antigens of the homologous organisms but not the living antigens. Formolised antigen A47, like the living antigen, failed to react with the two antisera, but formolised antigen A46 was agglutinated.

These results suggested that a heat-labile antigen may be present in living cells of actinobacilli. To test whether inactivation of the antigen occurred at the temperature at which the agglutination tests were incubated (56°C) several of the tests with living antigens and antisera against living organisms were repeated with incubation at 37°C. These results are also given in table VII which shows that, particularly with living antigen A49, incubation at the lower temperature gave a marked increase in the titre.

Absorption of antiserum A47 (living) with a living suspension of strain A49 resulted in the complete removal of antibodies for living antigens A47 and A49 although the titre for heated antigen A47 remained the same. On the other hand, absorption of this antiserum with a heated suspension of strain A47 removed antibodies for the heated antigen A47 but left the titres against living antigens A47 and A49 unchanged.

Attempts by microscopy to demonstrate capsules surrounding the cells of A. lignieresi proved unsuccessful but with a number of strains

there was some evidence of the presence of extracellular slime. Six strains producing this material were examined for the presence of extracellular antigens by means of the precipitation test. Two antisera against living A. lignieresi suspensions were employed in these tests, the results being shown in table VIII. Although the two immunising strains are of the same antigenic type (1) the saline extract from strain A3 (type 2) gave a positive precipitation reaction with antiserum A19. Furthermore the extract from strain A133 was tested against a second A133 antiserum prepared using a heat-killed immunising suspension. No precipitation occurred with this serum.

## DISCUSSION

The existence of a number of antigenic types of Actinobacillus lignieresi has been suspected for a considerable time. Thompson (1933) tested 15 strains of the organism from cattle and showed that there was variation in their antigenic structure but that cross-agglutination occurred with all of them. Tunnicliff (1941), most of whose strains were of ovine origin, was able to show one main antigenic group, but many of his strains did not fit into this group. Antigenic differences in ovine strains were also observed by Taylor (1944). In their investigation of 26 bovine strains, Till and Palmer (1960) came to the conclusion that there were at least two serological types of A. lignieresi and that the majority of their organisms belonged to one type.

Previous workers have employed agglutinable suspensions and antisera prepared in a number of ways. Davies and Torrance (1930), Jowett (1931)

and Taylor (1944) used living vaccines to prepare their antisera, whilst Tunnicliff, and Till and Palmer immunised with heat-killed bacteria and Thompson immunised with formaldehyde-killed bacteria. Agglutinable suspensions consisting of living organisms (Taylor) and heat-killed organisms (Till and Palmer) were used to demonstrate antigen-antibody union. No one worker, however, has made a comparison of the different types of antigen or antisera. The results with living and formolised antigens in the present work point to the existence of an envelope antigen that appears to be relatively non-specific, since cells associated with it are agglutinated by antisera against living organisms of unrelated heat-stable-antigen types. Such an envelope antigen may have been responsible for the results that led Davies and Torrance to the conclusion that their eleven recently isolated strains were identical.

Although no capsule was seen in wet-film India ink preparations, the presence of extracellular slime may well be linked with the presence of a submicroscopic capsule ("micro-capsule") that could bring about inagglutinability of living cells in tests with antisera prepared against the more deep-seated heat-stable antigens. The results obtained with formolised antigen A46 tested against antisera A46 (heated) and A47 (heated) suggest that the envelope antigen may not always be so well developed as to cause absolute inagglutinability of the cells. Variation in the amount of the envelope antigen could be expected to occur with repeated subcultivation since it is recognised that the viscous character of the colonies of A. lignieresi may be lost after several subcultures (Phillips, 1960).

The clear division of actinobacilli into types on the basis of their heat-stable antigens suggests that the major antigens concerned are quite distinct. The results with antisera A13, A47, A49 and A4 provide evidence, however, that there are minor antigens that may produce cross-agglutination reactions.

Some evidence of host specificity is shown with antigenic types 3 and 4 since, although the number of ovine strains of A. lignieresi included in this work is small, these two types are represented solely by organisms derived from sheep. The most commonly occurring antigenic type in the organisms from bovine sources is type 1 which, together with subtype 1a, comprises 66.0 per cent. of the total sample and 74.2 per cent. of the bovine strains. Although the origin of this collection of A. lignieresi strains is relatively restricted, being derived almost entirely from animals slaughtered at Edinburgh Abattoir, it is interesting to note that the National Collection of Type Cultures strains, all of which, Dr. S. T. Cowan has informed me, are of bovine origin, also fall into types 1 and 4a. The two type-1 strains (4975 and 4976) together with one of the subtype 4a strains (4985) were isolated in this country, and strains 4189 and 4191 were isolated by Thompson in America.

## SUMMARY

The antigenic structure of 218 strains of Actinobacillus lignieresi, isolated from lesions of actinobacillosis in cattle and sheep, was investigated by slide and tube agglutination tests and absorption tests.

Six antigenic types (no. 1 - 6) and two subtypes (1a, 4a) of organisms were distinguished by differences in their heat-stable antigens; 203 of the strains were classified in these types and 15 were untyped.

The majority of strains isolated from cattle belonged to type 1 and most of those from sheep to types 2, 3 and 4.

Heat-labile antigens common to different antigenic types were found in living and formaldehyde-killed organisms. These antigens may be responsible for inagglutinability of living organisms tested with antisera to the heat-stable antigens. The heat-labile antigenic material appeared to be associated with extracellular slime produced in small amounts by the organisms.

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TABLE I

Agglutination reactions between the heated bacterial antigens of 28 strains of Actinobacillus lignieresi and the antisera prepared against these antigens

| Antigen type | Antigen strain no. | Antiserum to heated antigens of strain no. | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 | A3 | A5 | A6 | A14 | A7 | A8 | A33 | A53 | A13 | A43 | A59 | A75 | A15 | A17 | A49 | A20 |
| 1 | A1 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |
| | A2 | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A4 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A19 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A22 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A46 | + | + | + | + | + | + | - | + | + | + | + | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - |
| | A47 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A55 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A56 | + | + | + | + | + | + | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A60 | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A100 | + | + | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A133 | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | A3 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | + | - | - | - | - | + | - | - | - | - | - | + | - |
| | A5 | - | - | - | - | - | - | + | - | - | - | - | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - | + | - |
| 3 | A6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | A14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | A7 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| | A8 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| | A33 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| | A53 | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | - | - | - | - | + | + | - |
| 5 | A13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| | A43 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| | A59 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| | A75 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | + | - |
| 6 | A15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | + | - | - |
| | A17 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | + | + | + | - |
| NST | A49 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | + | - |
| | A20 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + |

\+ = agglutination

**+** = agglutination by homologous antiserum

\- = no agglutination

NST = no standard type

TABLE II

Typing of 218 strains of A. lignieresi by patterns of slide agglutination reactions of heated bacterial antigens

| Type or subtype | Antisera causing agglutination | No. of strains in type | | |
|---|---|---|---|---|
| | | Strains from cattle | Strains from sheep | Strains from both sources |
| 1 | A1, A2, A4, A19, A22, A46, A47, A55, A56, A60 | 119 | 0 | 119 |
| 1a | As in type 1 but one or two of the sera failed to agglutinate | 25 | 0 | 25 |
| 2 | A3, A5 | 5 | 8 | 13 |
| 3 | A6 | 0 | 6 | 6 |
| 4 | A7, A33, A53 | 0 | 7 | 7 |
| 4a | As in type 4, but A8 or A33 failed to agglutinate | 2 | 1 | 3 |
| 5 | A13, A43, A75 | 21 | 0 | 21 |
| 6 | A15, A17 | 9 | 0 | 9 |
| Untyped | A4 only | 11 | 0 | 11 |
| Untyped | None | 2 | 2 | 4 |
| Total | | 194 | 24 | 218 |

TABLE III

Agglutination titres of antisera to heated antigens of twelve strains of type 1 before and after absorption with heated suspensions of the different organisms

| Antiserum to strain | Titre of unabsorbed antiserum in test with suspension of strain | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 |
| A1 | 160 | 160 | 80 | 160 | 80 | 160 | 80 | 160 | 160 | 80 | 80 | 160 |
| A2 | 80 | 80 | 80 | 80 | 40 | 80 | 40 | 80 | 80 | 40 | 80 | 80 |
| A4 | 40 | 40 | 80 | 80 | 40 | 40 | 40 | 40 | 40 | 40 | 80 | 80 |
| A19 | 80 | 40 | 80 | 80 | 40 | 80 | 40 | 40 | 80 | 80 | 80 | 80 |
| A22 | 2560 | 2560 | 2560 | 2560 | 2560 | 1280 | 2560 | 2560 | 5120 | 1280 | 2560 | 1280 |
| A46 | 1280 | 2560 | 1280 | 2560 | 1280 | 2560 | 1280 | 2560 | 2560 | 1280 | 1280 | 1280 |
| A47 | 80 | 80 | 160 | 160 | 80 | 160 | 160 | 80 | 160 | 80 | 40 | 80 |
| A55 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 40 | 20 | 20 | 20 | 20 |
| A56 | 80 | 80 | 160 | 160 | 80 | 160 | 80 | 80 | 160 | 80 | 80 | 80 |
| A60 | 160 | 160 | 320 | 160 | 80 | 320 | 160 | 160 | 160 | 320 | 160 | 160 |
| A100 | 1280 | 640 | 1280 | 1280 | 640 | 1280 | 640 | 1280 | 1280 | 640 | 2560 | 1280 |
| A133 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 80 | 160 |
| | Titre of antiserum in test with its homologous strain after absorption with strain | | | | | | | | | | | |
| | A1 | A2 | A4 | A19 | A22 | A46 | A47 | A55 | A56 | A60 | A100 | A133 |
| A1 | ... | 10 | N | N | N | N | N | N | N | N | N | N |
| A2 | N | ... | N | N | N | N | N | N | N | N | N | N |
| A4 | N | N | ... | N | N | N | N | N | N | N | N | N |
| A19 | N | N | N | ... | N | N | N | N | N | N | N | 10 |
| A22 | N | N | N | N | ... | N | N | N | N | N | N | N |
| A46 | 10 | N | N | N | N | ... | N | N | N | N | N | 20 |
| A47 | N | N | N | N | N | N | ... | N | N | N | N | N |
| A55 | N | N | N | N | N | N | N | ... | N | N | N | N |
| A56 | N | N | N | N | N | N | N | N | ... | N | N | N |
| A60 | N | N | N | N | N | N | N | N | N | ... | N | 40 |
| A100 | N | N | N | 20 | 10 | N | 10 | N | N | 10 | ... | 80 |
| A133 | N | N | N | N | N | N | N | N | N | N | N | ... |

N = no agglutination at a dilution of 1 in 10.

## TABLE IV

Agglutination reactions with antisera A13 and A47 before and after absorption with heated antigens of strains of A. lignieresi that were agglutinated in slide tests

| Antiserum to strain | Titre of unabsorbed antiserum in test with suspension of strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A3 | A5 | A7 | A13 | A17 | A20 | A33 | A47 | A49 | A53 |
| A13 | 40 | 20 | 10 | 2560 | 10 | 80 | 10 | ... | 20 | 10 |
| A47 | 80 | 40 | 10 | ... | ... | ... | 20 | 160 | ... | 20 |
| | Titre of antiserum in test with its homologous strain after absorption with strain | | | | | | | | | |
| | A3 | A5 | A7 | A13 | A17 | A20 | A33 | A47 | A49 | A53 |
| A13 | 2560 | 2560 | 2560 | ... | 2560 | 2560 | 2560 | ... | 2560 | 2560 |
| A47 | 80 | 40 | 80 | ... | ... | ... | 80 | ... | ... | 80 |

TABLE V

Slide agglutination reactions of portions of antiserum to heated antigen of strain A13 absorbed with four other strains of A. lignieresi in tests against slide antigens of eleven strains

| Antiserum A13 (type 5) absorbed with strain | Reaction with slide antigen of strain (type) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A3 (2) | A5 (2) | A7 (4) | A33 (4) | A53 (4) | A17 (6) | A20 (NST) | A49 (NST) | A13 (5) | A43 (5) | A75 (5) |
| A75 (type 5) | - | - | - | - | - | - | - | - | - | - | - |
| A5 (type 2) | - | - | - | - | - | - | - | - | + | + | + |
| A7 (type 4) | - | - | - | - | - | - | - | - | + | + | + |
| A17 (type 6) | - | - | - | - | - | - | - | - | + | + | + |

+ = agglutination

- = no agglutination

NST = no standard type

TABLE VI

Agglutination reactions of antisera to living and heated bacterial antigens in slide tests with living and heated antigens

| Antigen (agglutinable suspension) | | Reaction with antigen given by antiserum prepared against | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Living antigen of strain (type) | | | | Heated antigen of strain (type) | | | |
| Strain | Preparation | A5 (2) | A6 (3) | A7 (4) | A15 (6) | A1 (1) | A2 (1) | A3 (2) | A33 (4) |
| A1 | Living | + | + | + | + | - | - | - | - |
| (type 1) | Heated | - | - | - | - | + | + | - | - |
| A3 | Living | + | + | + | - | - | - | - | - |
| (type 2) | Heated | + | - | - | - | - | - | + | - |
| A7 | Living | + | + | + | - | - | - | - | - |
| (type 4) | Heated | - | - | + | - | - | - | - | + |

+ = agglutination

- = no agglutination

## TABLE VII

Agglutination titres of six antisera prepared against living and heated suspensions of three strains of A. lignieresi and tested against heated, formolised and living suspensions of the immunising strains

| Antigen (agglutinable suspension) | | Titre of antiserum prepared against | | | | | |
|---|---|---|---|---|---|---|---|
| | | heated organisms of strain | | | living organisms of strain | | |
| Strain | Preparation | A46 | A47 | A49 | A46 | A47 | A49 |
| A46 (type 1) | Heated | 1280 | 160 | N | 2560 | 5120 | N |
| | Formolised | 1280 | 160 | N | 5120 | 2560 | 80 |
| | Living | 10 | 10 | N | 640 (1280) | 640 | 40 (1280) |
| A47 (type 1) | Heated | 640 | 80 | N | 1280 | 2560 | N |
| | Formolised | N | 10 | N | 640 | 1280 | 160 |
| | Living | N | N | N | 640 | 640 (640) | N (640) |
| A49 (NST) | Heated | N | N | N | N | 10 | N |
| | Formolised | N | N | N | 320 | 320 | 160 |
| | Living | N | N | N | 10 (1280) | 10 (1280) | 40 (1280) |

Titres are the highest dilution of antiserum giving visible agglutination of the test strain of actinobacillus. Figures in brackets are the titres obtained when tests were incubated at 37°C instead of 56°C.

N = no agglutination at a dilution of 10 (i.e. 1 in 10).

NST = no standard type.

TABLE VIII

Precipitation reactions between saline extracts of six strains of A. lignieresi and two antisera prepared against living suspensions

| Antiserum prepared against strain | Reaction with saline extract prepared from strain | | | | | |
|---|---|---|---|---|---|---|
| | A3 | A15 | A6 | A7 | A19 | A133 |
| A133 | - | - | - | - | + | + |
| A19 | + | - | - | - | + | - |

+ = precipitation,

- = no precipitation.